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TRANSFORMATION OF THE SELF: A PATH TO REDEMPTION IN ALICE WALKER'S *THE THIRD LIFE OF GRANGE COPELAND*

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ABSTRACT

Alice Walker emphasis on the transformation of the self along with the changes in the society is explicitly depicted in her works. The protagonist and the important characters undergo phenomenal change, an inevitable change to better the status of the black men and women. The discrimination of the black by the white society can be obliterated only by the mutual compatibility of the black men and women. Women characters transform from passive and submissive nature to active and rebellious. She believes in the possibility of men becoming androgynous as well. Walker's conviction that emancipation of women is possible only when men mellow and transform themselves, is exemplified in her debut novel *The Third Life of Grange Copeland.*

Keywords: Alice Walker, The third life of Grange Copeland.

Alice Walker feels that the liberation of women is attainable only when men start understanding the sufferings of women as their own; when men change or reform or be responsible for the liberation of the entire race without excluding women from their fight for liberation;when "New man", put love in front as Christ, as she explains it in her poem, "The Abduction of Saints." In her interview with Winfrey, she told, "There is no heaven. This is it. We're already in heaven, you know, and so in order… for the earth to survive, we have to acknowledge each other as part of the family, the same family, and also reaffirm those things in ourselves and in other people that we've been brought up to fear or to hate(qtd. in Winfrey)."

According to Walker, the society is invariably a factor which causes change in a person and therefore the change should simultaneously happen both to the person and to the society. The struggle for liberation of the community and the individual should be in juxtaposition to make it whole. Precisely, the change should be both inner as well as outer. The black person thinks that he is not capable of being responsible for any of his actions or behaviour, because he thinks that only white folks are to be blamed for all his reactions and change. The black people should shed shame and develop self-love to accept responsibility of their own identity, their own lives instead of blaming the white folks and white society for their plight.

In Walker's novels, the men redeem themselves and grow. They turn inward to analyze the moral in their lives. Though they were subservient and powerless, after redemption they grow powerful and shed their slavery to gain their identity and individuality. Men especially mellow and save their internal morality and dignity, autonomy and balance, integrity and honour. Walker's conviction that emancipation of women is possible only when men mellow and transform themselves, is exemplified in her debut novel *The Third Life of Grange Copeland.* Men undergo metamorphosis. Copeland comes back from his "second life" in New York, as a new responsible man and a loving grandfather. Brutality of men is due to their incapability. The subservience, subjugation and powerlessness they suffer under the whites make them stretch themselves to the furthest point of male dominance and brutality. The victimizer is himself the victim of the white. Harold Bloomsays, "For the most part, the Black men in Walker's world are in need of redemption from the racism, oppression, and sexism still rampant in our society. They are in need of liberation from the near-zero images of themselves which has been propagated through the literature and the culture" (Bloom 17).

Walker is sympathetic toward her black male characters as they grow older. Her images of young black male brutality toward women are not surprising; violence was a fact of life in Eatonton in general and in her own family in particular. In an interview with David Bradley, Walker recalls,

I knew both my grandfathers, and they were just doting, indulgent, sweet old men. I just loved them both and they were crazy about me. However, as young men, middle-aged men, they were… brutal. One grandfather knocked my grandmother out of a window. He beat one of his children so severely that the child had epilepsy. Justa horrible, horrible man. But when I knew him, he was a sensitive, wonderful man *(www.nytimes.com)*

Her critics charged her with presenting a grossly negative image of Black men, who were portrayed as mean, cruel, or violent, entirely without redeeming qualities. Walker's criticism of black men is not a sign of enmity but a love for the well-being of the common humanity. Her futuristic outlook is seen in her desire to bring harmony between men and women by improving human character.

Walker's*The Third Life of Grange Copeland,* signifies that Grange Copeland, the protagonist, has three lives and it covers the three generations of the Copeland family and a period of American history from the 1920s to the 1960s. Grange's first life is dominated by his response to an oppressive, dehumanizing social structure which deprives him of his personhood and causes him to abuse his wife Margaret and to deny parental love and care to his son, Brownfield. The novel opens, in Grange's "first life" with his wife Margaret and their only child, Brownfield, in a run-down shack owned by the white man for whom he works "planting, chopping, poisoning, and picking in the cotton field" (Walker 7). In the novel, the family is permanently indebted permanently to the white owner of the cotton fields. Grange in his frustration finds vent to his subservience in drinking, in battering, in ignoring his wife and in being devoid of any emotion.

During the dreary cycle of the Copeland's weekly life together, Grange takes on animalistic traits. He spends the early part of each week recovering from the weekend's drunken splurge. By Thursday, however, the gloom of his situation has overtaken him once more and, animal-like, he stalks the house and swings from the rafters of the porch. By Friday, he is in a stupor from the work and the sun. On Saturday Grange cleans up and escapes down the road into town and into the arms of his lover, the prostitute Josie, staggering home later Saturday night "lurching drunk, threatening to kill his wife and Brownfield, stumbling and shooting off his shot gun" (Walker 12),while his wife, Margaret, and his son terrorized, hide in the woods.

On Sunday morning, he goes to church and raises "his voice above all the others… in song and in prayer" (Walker 13) and by nightfall both Grange and Margaret fight again. Monday morning he woke to start the cycle again. During the week, Whenever Grange happens to meet Shipley, the white man in whose field he labours, he sheds off his human traits. Walker tries to depict them through the images of masks and stones. When Shipley arrives in his truck at the end of the workday to collect the picked cotton, Brownfield had witnessed his father's freeze and he becomes a man of silence: For when the truck came his father's face froze into an unnaturally bland mask, curious and unsettling to see. It was as if his father became a stone or a robot. A grim stillness settled over his eyes and he became an object, a cipher, something that moved in tense jerks if it moved at all." (Walker 8)

The ascertained definition of manhood in African American faction as anywhere in the world, the yardstick to prove the worth and value of a man, is to take care of his self and his family and take up the responsibility of his wife and his children. But, Copeland has to live in cowardice and fear of a system; he has to identify himself by the definition of self by others. He breaches the definition of manhood in abandoning his family. He feels relieved about shedding his duties as a father, as a husband and as a breadwinner and finally fails to fit into the expected definition of manhood. He fails to complete the duties and responsibility of a man in a family and he fails to be a man in the society. He does not fulfill the requirements of a complete man because he fails to shoulder the responsibility expected of him. Resigned to his inability to control his own life or that of his wife and son, Grange contemptibly walks away. His first life ends with this desertion for which he got his just deserts.

Throughout the novel, Walker illustrates her own vision of the nature of the soul in transformation. That belief in the possibility of transformation at once makes the individual wholly responsible for his own salvation.Walker believes that a person is not a static product of the environment, but has the possibility of converting to different "lives" generated by transformations of the self. The novel offers a vivid illustration of a man who goes on to transform his life twice until he becomes a "reborn man", one who has achieved "a total triumph over life's misfortunes" (Walker 36). His first reduces him to the level of a "stone or a robot (Walker 8), he is a flaccid and unreceptive man of the environment because his loses his dignity gradually by the treatment he receives from his White master. Since he has lost his self-respect, he is not able to presume himself neither as a responsible husband or a father. Eventually, he abandons his family and goes to North. His experiences in the North, make him,alive and liberated for the first time in his life. Even though he indulges himself in vicious activities, for the first time he does something on his own accord, not dictated by a white system. Grange experiences "a new life" when he feels that he has played a role in the death of the white woman who drowned in Central Park Lake. As Robert Butler opines, "He acquires the strong conviction that such an act of murder has helped him to recover his manhood and self-respect (Walker 153) because it is

an act of rebellion against an environment intent on morally paralyzing him" (Walker 354).

In New York City, he enters a second stage of rage and rebellion. He reflects on the incident that propels him away from misery and fear towards hate. He watches a young pregnant white woman drown in a pond in a park. He watches the woman being rebuffed by her soldier lover and feels sympathy for her. Witnessing her leave the soldier's money and ring on the ground, he decided to help her by restoring the ring and part of the money, made bold by her pitiable state. But he tries to rescue her from drowning, the woman rejects him, Grange understands the power of rage. As she curses and insults him, he realizes the profundity of his own hatred towards the white. He hated the entire white race. His hatred is imposed by the misery of his life. When she refuses his hand, and drowned, he felt that his unfortunate life is repaid. He felt that he want to live again. After this incident, Grange makes his hatred of whites "his new religion" (Walker 153) and he strongly feels that the "hatred for the whites will someday unite us"(154). But this hatred gives only a false notion about liberation. He forgets the truth that he is the one who is responsible for his wife's suicide, he blames the white of all his problems. He gets rid of the guilt of abandoning his son Brownfield. So he fails to redeem him from the hatred which poisoned his life in the South. He once again becomes "a demon of hate and destruction"(Walker 136).

After leaving the park, he runs through the streets of New York, yelling, "Teach them to hate, if you want them to survive"(Walker 219). He is inflamed by his new found hatred and wants to inspire his fellow oppressed neighbours to hate as well, so that they all may live again. He tries to physically fight each white man he meets and to continue to encourage other African Americans to fight back as well. But he soon realizes that this sort of liberation is not possible because one man alone cannot swerve a community of oppressed people.

After this transformation he tries to make amends for his mistake. He realizes how his desertion of his family has resulted in the suicide of his wife Margaret and tries to help Brownfield's wife Mem. He realizes that racism has impoverished his life and made him guilty of the violence he inflicted on his own family and his child. He perceives that wounds would be healed and redemption is possible only when he admits his sins. He tells his son, "We guilty, Brownfield, and neither one of us is going to move a step in the right direction until we admit it"(Walker 209). When he admits that he allowed his wife to die and his family to fall apart, he truly takes human responsibility for his past actions. In this way he redeems himself to a radically new life. Butler observes, Grange undergoes a true conversion which genuinely transforms him, because it is only after he returns to Georgia that he recovers his place in a real community, which regenerates his ability to love and allows him to take full moral responsibility for the "sins" which have marred his first two lives (355).

His survival becomes more meaningful when he tried to help Brownfield's family by giving food and money. While Brownfield loses his humanity in the same Grange lost it in his first life, Grange recovers a human self by assuming familial roles which Brownfield discards. He treats Mem with kindness and helps her deliver Ruth on Christmas Day. From the moment, Grange dedicates himself to rescuing her and providing her with a viable family life. Love for Ruth is the fundamental factor responsible for converting Grange to a blossoming new life. "Whereas in his first life he was "smothered" (Walker 9) by dehumanizing society and in his second life he was "frozen" (Walker 145) by his demonic hatred of whites, he now becomes a "reborn man", redeemed by "love"(157). Through the magic of Ruth's miraculous hugs and kisses, he gradually overcomes his suicidal depression. He redeems himself from the numbness which had incapacitated Grange in his two lives through Ruth's redemptive power of love. He nurtures her, protects her, provides her education and educates her with the wisdom of his life's experiences, reeducating himself. An absolute transformation from a life of hatred to a life of love comes over him. Grange's life become whole and complete when he ensures Ruth the spiritual freedom.

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TEACHING LANGUAGE THROUGH TECHNOLOGY

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ABSTRACT

Teaching is demanding, exciting and gratifying. Students arrive in the classroom to learn and the task of the teacher becomes easier. Maintaining motivation then becomes the main objective. Brian O'Connell says "Without motivation, learning doesn't take place." Before teaching a class, it is essential to prepare and collect enough materials to cover the allocated teaching time. Teaching materials are used to promote learning, to maintain interest, to add variety to the lesson and to relate one subject to other subjects. There is a vast range of materials for teachers to use, although some are more readily available in certain countries than in others. Teachers always need more and more materials to keep up to date, and to renew worn items. It is useful to be aware of the applications of Modern Technology in the classroom although it is not readily available in many colleges or not available at all. Students enjoy variety in classroom and welcome the introduction of any form of film and screen presentations. The novelty factor immediately gains the full attention of the individual. And a highly professional studio production almost guarantees interest that will be maintained throughout the screening. Thus teaching through Technologies becomes a pleasurable experience for the students to learn. Modern appliances like Computers, Projectors, CD Player can be used in teaching. But all kind of Technologies still need the presence of a teacher, his smile, his heart and his motivation. The aim must be to produce a new generation full of humanity and life and not Computer – like students.

Keywords: Teaching language, Modern technology.

A Teacher's aim is to have the satisfaction of doing a good job, of creating learning situations for the maximum benefit of the students. To succeed in teaching, a teacher must strive to hold the attention of the students and make the class interesting. Teaching materials promote learning, maintain interest, add variety to the lesson and relate one subject to the other subjects. Hence it is essential for teachers to prepare and collect enough materials to teach. It is always a wise precaution to have extra material in classroom. There is a vast range of materials readily available for teachers to use.

Introducing materials in classroom enhance teaching and help to keep the students interest alive. Sitting and listening to a lecture becomes boring and the teacher has to strive for variety in teaching methods and materials. It is useful to be aware of the applications of modern technology in the classroom. During the second half of the twentieth century, modern technology was used to support language and learning. Technological devices are useful to teachers who wish to bring sounds and sights into the class room. Students enjoy variety within the classroom and welcome the introduction of any film and screen presentation. The novelty factor immediately gains the entire attention of the individual and a highly professional studio production almost guarantees interest that will be maintained throughout the screening. The students learn interestingly during this pleasurable experience and sufficient time must be allowed to the students to have discussion after screening or home assignments can be given based on it. Catalogues are available in libraries enumerating a list of educational films and cassettes available

for the students. Hence it is the task of a teacher to update her knowledge and expertise by a wide reading of the latest developments in teaching.

Adequate facilities like good classrooms, audio-video aids, congenial atmosphere etc go a long way in making the teaching purposeful and interesting. A lot of high quality audio materials are available for learners. They bring authentic voices into the classroom and make the learner to listen repeatedly to the most appropriate forms of spoken language. Video display provides visual support, variety and entertainment. Videos stimulate learner participation in role play and discussion. The most versatile and the most powerful educational aid and material is Computer, which offers sizeable contribution to language teaching. Computer is a powerful tool to store, transmit, present, retrieve and sort large amount of information in a non-linear way. Computer- based education is usually divided into Computer –Assisted Instruction and Computer – Managed Instruction. Computer–Assisted Instruction (CAI) generally emphasis the complementary role of a computer in the teaching or learning process, even if computers are employed to provide instruction

directly to learners. In early 1960s, large mainframe computers were used by a small number of University Language educators. But in the mid –to-late 1980s, an increasing number of language teachers became computer literate and started using computers while planning and carrying out their teaching. Power point presentation and other computer programs improve teacher's presentation of materials to the class.

The integration of information technology in teaching is a central matter in ensuring quality education. There are two important reasons for integrating information technology in teaching. Pupils must have familiarity with the use of information technology, since all jobs in the society of the future will be dependent on it and information technology must be used in teaching in order to improve its quality and make it more effective. Technology has become an important component in the current age. Every day the introduction of some new gadget and software make lives easier and improves the technology and software that already exists. Technology is playing an important role in education. As technology, advances it is used to benefit students of all ages in the learning process. Technology in classroom helps students absorb the material. Projection screen linked to computers allows students to see their notes instead of simply listening to a teacher's lecture.

The use of technology changes teaching in several ways. Technology makes teachers to be more creative in customizing their own material power. Students accept using technology for granted and the teachers must be expert in the use of technology to get students involved in the process of learning. Technology makes teaching easier and better. But it must be remembered that the new technologies will become old tomorrow and new things will come to life to bury the tools of today. However all technologies and tools will be appreciated as long as they satisfy the students thirst for knowledge .Most teachers generally feel that the introduction of technology may result in the loss of control, but, it must be remembered that the technological tools are not monsters and that a resourceful teacher can never be replaced.

There is no hard and fast rule as the right way to teach and this is a kind of blessing. But the day such a thing is discovered, will be the day that teaching becomes very dull. Teaching the student is a challenge and teachers seek and strive for innovative ways enabling learning more effective. This search for improvement makes teaching alive. It is not a matter how successful a course has been, but with happy students and good results a teacher should be looking ahead and considerable possible changes has to be made to the next course. While teaching a course, ideas often occur that perhaps next time a different approach may be followed. Perhaps there may be something discussed with colleagues or at conferences or read, that suggests some new ideas of teaching a course. An open mind, willingness to change when the need for change is obvious, will keep the teachers up to date and in the mainstream of teaching techniques.

Students remember an outstanding teacher from their own school days. Students who become teachers may be impressed by a particular teacher whom they observe in the course of their training. But in teaching one should be always be oneself. No book can totally prepare one for teaching. One has to find one's own way in teaching. It is a search for one's own salvation. Systems and ideas can only help teachers. Buddha said "Be a light unto yourself ". (Krishnaswamy 342). The search is a never ending process in the evolution of a teacher.

A Teacher has to know her materials well, to put them to good use. A knowledge about the good set of materials is essential. Equally essential is the studied view of how to draw upon sources other than prescribed materials and how to relate them effectively to the topic addressed at a particular stage. It is smart to look for ways that will improve the ability of the teachers to teach but it's hard to see a machine that replaces the role of the teacher. Teaching is a human experience. Modern appliances like Computers, Projectors, CD player can be used in teaching. But all kind of Technologies still need the presence of a teacher, his smile, his heart and his motivation. The aim must be to produce a new generation full of humanity and life and not computer – like students.

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CONTRA αgμ-CONTINUOUS FUNCTIONS

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ABSTRACT

In this paper we introduce and discuss some basic properties of contra αgμ-continuous functions.

Keywords : αgμ-open, contra αgμ-continuous

1. INTRODUCTION

The notion αgμ-closed sets in topological spaces was introduced by R. Devi, V. Vijayalakshmi and V. Kokilavani. The concept of Contra continuous mappings was introduced and investigated by J. Dontchev. In this paper we introduce the notion of contra αgμ-continuous functions and discuss their basic properties

2. PRELIMINARIES

2.1. Definition

A subset A of space (X,τ) is called

1. a generalized closed (briefly g-closed) set (Njastad,1965) if cl(A) ⊆ U and U is open in (X, τ); the complement of a g-closed set is called a g-open set,

2. an α-generalized closed (briefly αg-closed) set (Maki *et al.,*1994) if αcl(A) ⊆ U whenever

A ⊆ U and U is open in (X, τ),

3. a μ-closed set (Veera Kumar, 2005) if cl(A) ⊆ U whenever A ⊆ U and U is gα*-open in (X, τ),

4. an αgμ-closed set (Devi *et al.,* 2007) if αcl(A) ⊆ U whenever A ⊆ U and U is μ-open in (X, τ).

2.2. Definition

1. A space (X, τ) is said to be ${}_cT_{\alpha g\mu}$ (Devi *et al.,* 2007) if every αgμ-closed set is closed in X.

2. A space (X, τ) is said to be locally indiscrete (Atick, 1997) if every open subset of X is closed.

3. A function f : (X, τ) →(Y, σ) is said to be regular set connected (Atick, 1997) if $f^{-1}(V)$ is clopen in (X, τ) for every regular open set V of (Y, σ).

4. A function f : (X, τ) →(Y, σ) is said to be perfectly continuous (Atick, 1997) if $f^{-1}(V)$ is clopen in X for every open set V of Y.

2.3. Definition

A function f : (X, τ) → (Y, σ) is said to be contra continuous (Dontchev, 1996) if for every open set in (Y, σ) there exist a closed set in (X, τ).

2.4. Definition

A function f: (X, τ) → (Y, σ) is said to be αgμ-continuous (Devi *et al.,* 2007) if for every open set in (Y, σ) there exist an αgμ-open set in (X, τ).

3. CONTRA αGμ-CONTINUOUS FUNCTION

3.1. Definition

A function f: (X, τ) → (Y, σ) is said to be contra αgμ-continuous if for every open set in (Y, σ) there exist an αgμ-closed set in (X, τ).

3.2. Theorem

Every contra continuous function is contra αgμ- continuous.

Proof. Let V be open in (Y, σ). Since f : (X, τ) → (Y, σ) is contra continuous, $f^{-1}(V)$ is closed in (X, τ) and hence αgμ-closed By (Devi *et al.,* 2007). Thus, f is contra αgμ continuous.

Converse of the above theorem need not be true by the following example.

3.3. Example

Let X = Y = {a, b, c}, τ = {ϕ, X, {a}, {b, c}} and σ = {ϕ, Y, {a}, {a, b}}.

Define f : (X, τ) → (Y, σ) by f(a) = b, f(b) = c and f(c) = a. The αgμ-closed sets of X are ϕ, X, {a},{b}, {c}, {a, b}, {b, c}, {a, c}. Here {a, b} is an open set of (Y, σ) but $f^{-1}(\{a, b\})$ = {b, c} is not a closed set of (X, τ). Hence f is contra αgμ- continuous but not contra continuous.

3.4. Lemma

The following properties hold for subsets A,B of a space X:

(a) $x \in \ker(A)$ if and only if $A \cap F \neq \phi$ for any $F \in C(X,x)$.

(b) $A \subset \ker(A)$ and $A = \ker(A)$ if A is open in X.

(c) If $A \subseteq B$, then $\ker(A) \subseteq \ker(B)$.

3.5.Theorem

For a function $f : (X, \tau) \to (Y, \sigma)$ the following conditions are equivalent:

(1) f is contra αgμ- continuous;

(2) for every closed subset F of Y, $f^{-1}(F) \in \alpha g\mu O(X)$;

(3) for each $x \in X$ and each $F \in C(Y,f(x))$, there exists $U \in \alpha g\mu O(X,x)$ such that

$f(U) \subseteq F$;

(4) $f(\alpha g\mu cl(A)) \subseteq \ker(f(A))$ for every subset A of X;

(5) $\alpha g\mu cl(f^{-1}(B)) \subseteq f^{-1}(\ker(B))$ for every subset B of Y.

Proof.

(1) $\Rightarrow$ (2) Since f is contra αgμ- continuous, inverse image of a closed subset F of Y is $\alpha g\mu O(X)$.

(2)$\Rightarrow$(3) It is given F is closed subset of Y and $f^{-1}(F)$ is $\alpha g\mu O(X)$. Hence for $x \in X$, there exists $U \in \alpha g\mu O(X)$ such that $f(U) \subseteq F$.

(3) $\Rightarrow$ (2) Let F be any closed set of Y and $x \in f^{-1}(F)$. Then $f(x) \in F$ and there exists $U_x \in \alpha g\mu O(X,x)$ such that $f(U_x) \subseteq F$. Therefore, we obtain $f^{-1}(F) = \cup\{U_x / x \in f^{-1}(F)\}$ and $f^{-1}(F)$ is αgμ-open.

(2) $\Rightarrow$ (4) Let A be any subset of X. Suppose that $y \notin \ker(f(A))$. Then by the lemma 3.4, there exists $F \in C(Y,f(x))$ such that $f(A) \cap F = \phi$. Thus, we have $A \cap f^{-1}(F) = \phi$ and since $f^{-1}(F)$ is αgμ-open, we have $\alpha g\mu cl(A) \cap f^{-1}(F) = \phi$.

Therefore, we obtain $f(\alpha g\mu cl(A)) \cap F = \phi$ and $y \notin f(\alpha g\mu cl(A))$. This implies that $f(\alpha g\mu cl(A)) \subseteq \ker(f(A))$.

(4) $\Rightarrow$ (5) Let B be any subset of Y. By lemma 3.4, we have

$f(\alpha g\mu cl(f^{-1}(B))) \subseteq \ker(f(f^{-1}(B))) \subseteq \ker(B)$ thus $\alpha g\mu cl(f^{-1}(B)) \subseteq f^{-1}(\ker(B))$.

(5) $\Rightarrow$ (1) Let V be any open set of Y. Then by lemma 3.4, we have

$\alpha g\mu cl(f^{-1}(V)) \subseteq f^{-1}(\ker(V)) = f^{-1}(V)$ and $\alpha g\mu cl(f^{-1}(V)) = f^{-1}(V)$.This shows that $f^{-1}(V)$ is αgμ-closed in X.

3.6. Theorem

If a function $f: (X, \tau) \to (Y, \sigma)$ is contra αgμ-continuous and Y is regular, then f is αgμ-continuous.

Proof. Let x be an arbitrary point of X and let V be an open set of Y containing f(x). Since Y is regular, there exists an open set W in Y containing f(x) such that $cl(W) \subseteq V$. Since f is αgμ-continuous, so by theorem3.5, there exists $U \in \alpha g\mu O(X,x)$ such that $f(U) \subseteq cl(W)$. Then $f(U) \subseteq cl(W) \subseteq V$. Hence, f is αgμ-continuous.

3.7. Corollary

If a function $f: (X, \tau) \to (Y, \sigma)$ is contra αgμ-continuous and Y is regular, and then f is continuous.

We introduce the following definitions

3.8. Definition

1) A space (X, τ) is said to be locally αgμ-indiscrete if every αgμ-open set is

closed.

2) A function $f: X \to Y$ is called almost αgμ-continuous if for each $x \in X$ and each

open set V of Y containing f(x), there exists $U \in \alpha g\mu O(X, x)$ such that $f(U) \subseteq \alpha g\mu int(cl(V))$.

3.9.Theorem

If a function $f: (X, \tau) \to (Y, \sigma)$ is contra αgμ-continuous and X is a ${}_cT_{\alpha g\mu}$, then f is αgμ-continuous.

Proof. Let V be a closed set in Y. Since f is contra αgμ-continuous, $f^{-1}(V)$ is αgμ open in X. Since X is ${}_cT_{\alpha g\mu}$, $f^{-1}(V)$ is open in X. Hence f is contra-continuous.

3.10. Theorem.

Let X be locally αgμ-indiscrete. If $f : (X, \tau) \to (Y, \sigma)$ is contra αgμ-continuous, then f is continuous.

Proof. Let V be a closed set in Y. Since f is contra αgμ-continuous, $f^{-1}(V)$ is αgμ-open in X. Since X is locally αgμ-indiscrete, $f^{-1}(V)$ is closed in X. Hence f is continuous.

3.11. Theorem

A function $f : X \to Y$ is almost αgμ-continuous if and only if for each $x \in X$ and each regular open set V of Y containing f(x), there exists $U \in \alpha g\mu O(X, x)$ such that $f(U) \subseteq V$.

Proof. Let V be regular open set of Y containing f(x) for each $x \in X$. Since every regular open set is open

(Njastad, 1965), V be an open set of Y containing f(x) for each x ∈X.

Since f is almost αgμ-continuous, there exists U ∈αgμO(X, x) such that f(U) ⊆αgμint(cl(V))⊆ V. Therefore f(U) ⊆ V.

Conversely, if for each x ∈X and each regular open set V of Y containing f(x), there exists U ∈αgμO(X, x) such that f(U) ⊆ V. This implies V is an open set of Y containing f(x), such that f(U) ⊆ V =αgμint(cl(V)). Therefore f is almost αgμ-continuous.

3.12. Theorem

If a function f: (X, τ) → (Y, σ) is pre αgμ-open and contra αgμ-continuous, then f is almost αgμ-continuous.

Proof. Let x be any arbitrary point of X and V be an open set containing f(x).Since f is contra αgμ-continuous, then there exists U ∈αgμO(X, x) such that f(U) ⊆ cl(V). Since f is pre αgμ-open, f(U) is pre αgμ-open in Y. Therefore, f(U) = αgμint(f(U)) ⊆ αgμint (cl(f(U))) ⊆ αgμint (cl(V)). This shows that f is almost αgμ-continuous.

3.13. Definition

The graph of a function f: X→Y is said to be contra αgμ-closed if for each

(x, y) ∈ (X × Y) – Gr(f), there exists U ∈αgμO(X, x) and V ∈ C(Y, y) such that (U × V) ∩ Gr (f) = ϕ.

3.14. Theorem

If f: X →Y is contra αgμ-continuous and Y is Urysohn, then f is Cαgμ-closed in the product space X × Y.

Proof. Let (x, y) ∈ (X × Y) – Gr(f). Then y ≠ f(x) and there exists open sets A and B such that f(x) ∈A, y ∈ B and cl(A) ∩ cl(B) = ϕ. Then there exists V ∈αgμO(X, x) such that f(V) ⊆ cl(A). Therefore, we obtain f(V) ∩ cl(B) = ϕ. This shows that f is Cαgμ-closed.

3.15. Theorem

If f : X→Y is contra αgμ-continuous with X as locally αgμ-indiscrete then f is continuous.

Proof. Let V be an open set in Y. Since f is contra αgμ-continuous, $f^{-1}(V)$ is αgμ-closed set in X. Since X is locally αgμ-indiscrete every αgμ-closed set is open. Hence $f^{-1}(V)$ is open in X. Therefore f is continuous.

3.16. Theorem

If f: X→Y is contra αgμ-continuous and X is ${}_cT_{\alpha g\mu}$-space, then f is contra- continuous.

Proof. Let V be open set in Y. Since f is contra αgμ-continuous, $f^{-1}(V)$ is αgμ-closed in X. Since X is a ${}_cT_{\alpha g\mu}$ space, every αgμ-closed set is closed. Hence $f^{-1}(V)$ closed in X. Therefore f is contra- continuous.

3.17. Theorem

If f: X→Y is a surjective pre-closed contra αgμ-continuous with X as ${}_cT_{\alpha g\mu}$ space, then Y is locally indiscrete.

Proof. Let V be an open subset in Y. Since f is contra αgμ-continuous, $f^{-1}(V)$ is αgμ-closed in X. Since X is ${}_cT_{\alpha g\mu}$ space $f^{-1}(V)$ is closed in X. Since f is pre-closed,V is pre-closed in Y. Now we have cl(V) = cl(int(V)) ⊆ V which implies cl(V) = V. This means V is closed in X and hence Y is locally indiscrete.

3.18. Definition

A space X is said to be αgμ-connected if X cannot be written as a disjoint union of two non-empty αgμ-open sets.

3.19. Theorem

A contra αgμ-continuous image of a αgμ-connected space is connected.

Proof. Let f: X→Y be a contra αgμ-continuous map of a αgμ-connected space X on to a topological space Y. If possible, let Y be disconnected. Let A and B form a disconnection of Y. Then A and B are clopen and Y = A ∪ B, where A ∩ B = ϕ. Since f is contra αgμ-continuous map, X = $f^{-1}(Y) = f^{-1}(A \cup B) = f^{-1}(A) \cup f^{-1}(B)$ where $f^{-1}(A)$ and $f^{-1}(B)$ are non-empty αgμ-open sets in X. Also $f^{-1}(A) \cap f^{-1}(B) = \phi$. Hence X is not αgμ-connected. This is a contradiction. Therefore Y is connected.

3.20. Theorem

If f is contra αgμ-continuous map from a αgμ-connected space X on to any space Y, then Y is not a discrete space.

Proof. Suppose that Y is discrete. Let A be a proper non-empty open and closed subset of Y. Since f is contra αgμ-continuous, $f^{-1}(A)$ is a proper non-empty αgμ-open and αgμ-closed subset of X, which is a contradiction to the fact that X is αgμ-connected space. Therefore Y is not a discrete space.

3.21. Theorem

If f: X→Y is αgμ-irresolute map with Y as locally αgμ-indiscrete space and g : Y→Z is contra

αgμ-continuous map, then g_of: X→Z is αgμ-continuous.

Proof. Let A be any closed set in Z. Since g : Y→Z is contra αgμ-continuous, $g^{-1}(A)$ is αgμ-open set in Y. Since Y is locally αgμ-indiscrete, $g^{-1}(A)$ is closed in Y. Hence $g^{-1}(A)$ is αgμ-closed set in Y. Since f is αgμ-irresolute $(g_of)^{-1}(A)) = f^{-1}(g^{-1}(A)$ is αgμ-closed in X. Therefore g_of is αgμ-continuous.

3.22. Theorem

If g : Y→Z is continuous and f : X→Y is contra αgμ-continuous then g_of : X→Z is contra αgμ-continuous.

Proof. Let A be a closed set in Z. Since g: Y→ *Z* is continuous, $g^{-1}(A)$ is closed in Y. Since f is contra αgμ-continuous, $(g_of)^{-1}(A)) = f^{-1}(g^{-1}(A)$is αgμ-open in X. Thus g_of is contra αgμ-continuous.

3.23. Theorem

If f: X→Y and g: Y→Z are αgμ-continuous and Y is locally αgμ-indiscrete, then g_of : X→Z is contra αgμ-continuous.

Proof. Let A be a closed set in Z. Since g is αgμ-continuous, $g^{-1}(A)$ is αgμ-closed in Y and hence open. Since f is αgμ-continuous $(g_o f)^{-1}(A)= f^{-1}(g^{-1}(A))$ is αgμ-open in X. Hence g_of is contra αgμ-continuous.

3.24. Theorem

If f: X→Y is surjective αgμ-irresolute and preαgμ-open and g: Y→Z is any function, then g_of: X→Z is contra αgμ-continuous if and only if g is contra αgμ-continuous.

Proof. To prove if part, let g be contra αgμ-continuous and A be a closed set of Z.

Since g is contra αgμ-continuous, $g^{-1}(A)$ is αgμ-open in Y. Since f is αgμ-irresolute, $(g_of)^{-1}(A)= f^{-1}(g^{-1}(A))$ is αgμ-open in X. Hence g_of: X→Z is contra αgμ-continuous.

To prove only if part, let g_of: X→Z is contra αgμ-continuous and let A be a closed set in Z. Then $(g_o f)^{-1}(A)$ is αgμ-open of X. That is $f^{-1}(g^{-1}(A))$ is an αgμ-open subset of X. Since f is pre αgμ-open, $f(f^{-1}(g^{-1}(A)))$ is αgμ-open subset of Y. So, $g^{-1}(A)$ is an αgμ-open subset of Y. Hence g is contra αgμ-continuous.

3.25. Theorem

If f : X→Y is contra αgμ-continuous, closed injection and Y is ultra normal, then X is αgμ-normal.

Proof. Let A and B be disjoint closed subsets of X. Since f is closed injectve, f(A) and f(B) are disjoint closed subsets of Y. Since Y is ultra normal, f(A) and f(B) are separated by disjoint clopen sets V and W respectively. Hence A $\subseteq$ $f^{-1}(V)$ and B $\subseteq f^{-1}(W)$, $f^{-1}(V) \subseteq \alpha g\mu O(X)$ and $f^{-1}(W) \in \alpha g\mu O(X)$. Also $f^{-1}(V) \cap f^{-1}(W) = \phi$. Thus X is αgμ-normal.

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CONSTRUCTION OF MINIMUM CONNECTED DOMINATING SET IN A GRAPH AND ITS APPLICATION IN WIRELESS SENSOR NETWORKS

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ABSTRACT

Finding Minimum Connected Dominating Set in a graph is one of the important problems in graph theory. It is largely applied in Wireless Sensor Networks (WSN). It acts as a virtual backbone in WSNs. algorithms of finding such minimum connected dominating set was studied. A new algorithm for constructing a routing in WSN using one of the algorithms is proposed and implemented using java language. The sample outputs are also included.

Keywords: Connected Dominating Set, wireless sensor networks, virtual backbone.

I. INTRODUCTION

A backbone in a Wireless Sensor Network (WSN) reduces the communication overhead, increases the band width efficiency, decreases the overall energy consumption and thus increases network operational life. The nodes in a wireless sensor network forward data towards a sink via other nodes. The limited resources on the nodes require minimum energy to be spent in this energy consuming task. This necessitates a virtual backbone that can minimize the number of hops required to reach the sink assuming that all nodes have equal transmission range. In the wireless domain, this backbone is a minimum connected dominating set (MCDS). Away from an element of the subset forms a dominating set *S*. A connected dominating set (CDS) *C* of *G* is a dominating set *S* in hitch all the elements are connected *i.e.* it induces a connected graph. The nodes in *C* are called dominators and the other nodes which are one hop away from *C* are dominates. To minimize the number of hops, the minimum CDS is chosen as the backbone. The backbone is the smallest CDS and every node is adjacent to this virtual backbone. Once data is received by a dominator, it is relayed through the MCDS towards the sink for minimum hop communication. Since the nodes have equal transmission range, the CDS has to be determined for Unit Disk Graph.

2. AUXILIARY DEFINITION

In order to develop the algorithm, we state some definition and introduce some terminology relevant to the paper.

2.1. Dominating Set – Dominating Set for a graph G = (V, E) is a subset D of the Vertex Set V such that each vertex uÎV is either in D or adjacent to some vertex v in D. The elements of dominating set are called dominators. Examples of dominating set in a graph G are given below:

Figure 1. {1, 3}, {2, 3, 5} and {1, 2, 3, 4} are Dominating sets

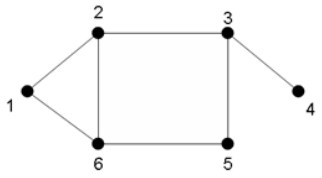
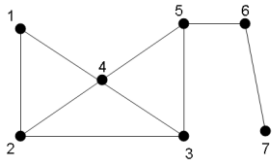


Figure 2. {4, 6}, {1, 5, 7} and {4, 5, 6} are Dominating Sets



2.2. Connected Dominating Set – A Connected Dominating Set (CDS) of a graph G = (V,E) is a set of vertices with two properties:

1. D is a dominating set in G.

2. D induces a connected subgraph of G.

In Fig.1, {2, 3, 5} and {1, 2, 3, 4} are Connected Dominating Sets. Similarly in Fig. 2, {4, 5, 6} is a Connected Dominating Set.

2.3. Minimum Connected Dominating Set – A minimum Connected Dominating Set (MCDS) is a connected dominating set with smallest possible cardinality among all the CDSs of G. As in Figs. 1 and 2, {2, 3, 5} and {4, 5, 6} are Minimum Connected Dominating Sets respectively.

2.4. Convex hull – the convex hull for a set of points X in real vector space is the minimum convex set

containing X. it is also called convex envelop and denoted by CH(X). It is represented by a sequence of the vertices of the line segment forming the boundary of the convex polygon.

3. A POWER AWARE MINIMUM CONNECTED DOMINATING SET FOR WIRELESS SENSOR NETWORKS.

A backbone in a wireless sensor networks (WSN) reduces the communication overhead, increases the bandwidth efficiency, decreases the overall energy consumption and thus increases network operational life. The nodes in a wireless sensor networks forward data towards data a sink via other nodes.

The limited resources on the nodes require minimum energy to be spent in this energy consuming task. This necessitates a virtual backbone that can minimize the number of hopes required to reach the sink assuming that all nodes have equal transmission range. In the wireless domain, this backbone is a minimum connected dominating set (MCDS).

3.1. Finding a Routing in WSN Using Power Aware Minimum Connected Dominating Set

Here, we are presenting an algorithm for a finding a routing in WSN using power aware minimum connected dominating set is presented. The proposed algorithm is divided into two phases. In the phase a random network is generated and in the second phase routing is achieved using the algorithm.

3.2. Generation of Random Network

Random graphs were introduced by erdos and renyi in the late fifties. The random graph model generates a graph that has a number has a number of nodes which are connected randomly by undirected edges. A random graph is obtained by starting with a set of n vertices and adding edges between them at random. Here a random nodes are initially placed in the specified area m x m and at each iteration edges are created randomly using those nodes.

3.3. MCDS Construction

Dominating set is constructed which consists of minimum number of nodes. This phase consists of following steps:

1. An arbitrary number say *id* is assigned to each Node in the graph $G(V,E)$

2. Each node is assigned white color

3. The node u with maximum degree is taken from $G(V,E)$ and colored as black, *i.e.* Dominator

4. All the neighbor nodes of the node u are Colored *i.e*. Dominatee

5. Do step 3-4 till all the nodes in the graph $G(V,E)$ are colored either as black or gray.

PHASE-I (G (V, E))

$D \leftarrow ¢$

if ($\forall x \in V$ x.color is black or gray)

then *exit*

else u ← MAX-DEGREE(V)

u.color ← *black*

$D \leftarrow D \cup \{u\}$ **for** $\forall x \in N \{u\}$

do x.color ← *gray*

Phase-1 Algorithm

PHASE -1 ((G(V,E))

Explanation of Algorithm Phase-I: Each node in the graph is assigned an arbitrary number as *id*. Each node is assigned with white color in the beginning. A node $x \in G$ (V, E) s.t. x has maximum degree is determined; if two nodes have same degree *i.e*. maximum then choose a node having minimum *id*. Let that node be u. color node u as black i.e. Dominator and this node is added into list of Dominating Set *i.e*. *D*. All the neighbors of node *u i.e*. Dominates are colored as gray so that they are not considered in dominating set. The same is repeated for remaining uncolored graph until all the nodes get colored. The above given algorithm can be understood with the help graph shown in figure 4. In this graph initially all nodes are considered as white nodes. Select node which has maximum degree. Node 8 and node 12 both have maximum degree *i.e.* five. according to this algorithm in case of tie of the degree, lowest *id* is considered first, so node 8 is selected and colored black and all its neighboring nodes *i.e* 6,7,9,10and 11 are colored gray.

Fig 3.1. Initial Graph

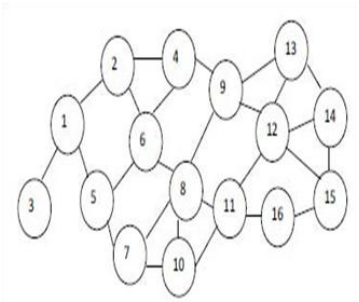
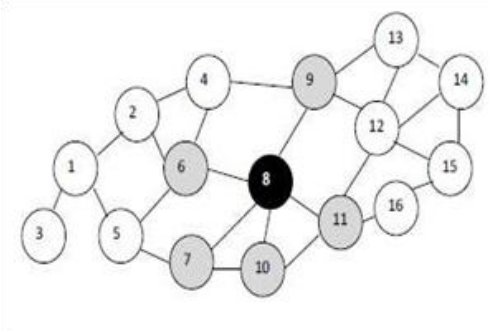
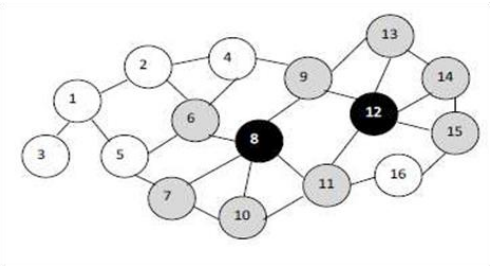


Fig.3.2 .Node 8 selected as domnting node



Similarly in next step node 12 is considered which has maximum degree and colored black and its neighbours i.e. 9,11,13,14 and 15 colored as gray.

Fig 3.3. Node 8 and 12 selected as dominating set



In next step same procedure repeat and node 1 is selected which has maximum degree with lowest *i.d.*, colored black and all its neighbors 2,3 and 5 colored as gray.

Fig 3.4: Nodes 1.8.12 selected as dominating set

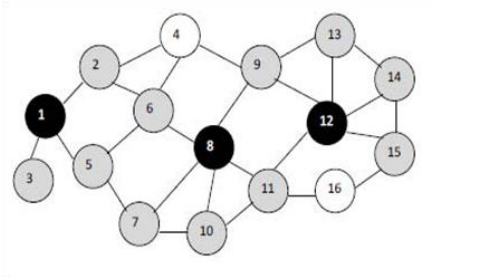
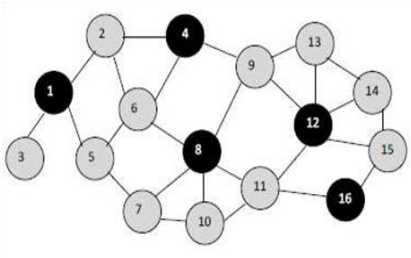


Fig 3.5. Nodes 1,4,8,12 and 16 are dominatig set nodes



Phase II. Determination of Connectors

In this phase, set of connectors B is found such that all the nodes in dominating set D gets connected. Let a black node be a node in D and a dark gray node represent a node in B. a node in B is connected by at most K nodes in the graph G (V, E). Set of dark gray nodes with given D could be found using Steiner tree. It is a tree, interconnecting all the nodes in D by adding new nodes between them. The nodes that are in the Steiner tree but not in set D are called Steiner nodes. In the MCDS set, the number of Steiner nodes should be minimum. After this steps CDS is constructed, which will consist of black and dark gray nodes. Let the constructed CDS be set F. This involves the following steps.

1. Select a gray node which is connected to Maximum (K) number of black nodes, set

Its color as dark gray

2. Check whether the Dominating Set D

3. if D gets connected stop

4. else go to step 1 with K–1 number of black nodes

Phase – II Algorithm

$B \leftarrow$ ¢

for $i \leftarrow$ Kto2

do

while there exist a node v which is connected to K adjacent black nodes

do

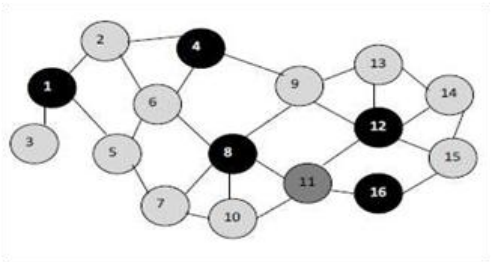
$B \leftarrow B \cup \{v\}$

if D is connected

then *exit*

after completing the dominating set nodes 1,4,8,12 and 16 select a node which connected maximum number of black nodes i.e. node 11 and colored s dark gray

Fig 3.6. Connecting node 11



Repeat this step until all black nodes are connected . All the black nodes and all the dark gray nodes are form the connecting dominating set.

Fig 3.7. Connecting nodes 11 and 9

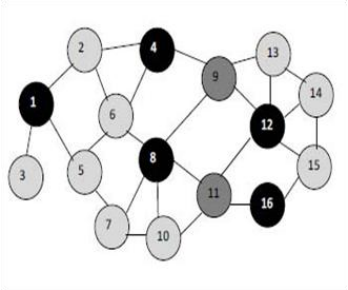
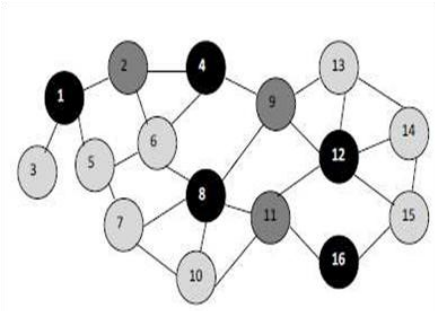
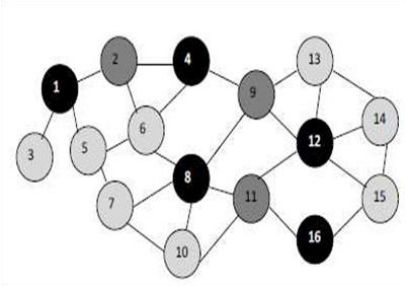


Fig 3.8. Connecting nodes 9,11 and 2



So final CDS which covers nodes 1,2,4,8,9,11,12 and 16. This can be shown in fig 3.9.

Fig 3.9: cds nodes are 1,2,4,8,9,11,12 and 16.



Phase III: Pruning

This is the pruning phase. In this phase, redundant nodes are deleted from the CDS constructed in phase II, to obtain the MCDS. The following steps are

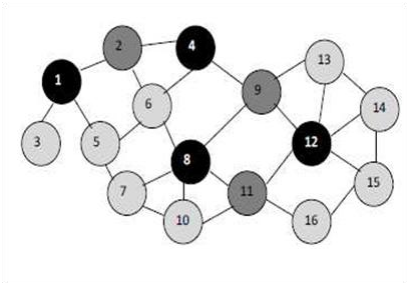
1. Select a minimum degree node *u* from *F*

2. check if *N*[*u*] is subset of *N*[1] and *N*[2] and ...*N* [*n*] where *i* belongs to *F* –{*u*}

3. if step 2 returns *true* then remove node *u* and Goto step 1

4. Otherwise do not remove node *u* and Goto step 1

Fig 3.10. {1,2,4,9,8,11,12} is finial MCDS backbone



For this CDS graph select a node with the minimum degree among black nodes and dark gray nodes and delete it. Node 16 is deleted because node 16 is subset of N (12) N (11). The degree of node 16 is less than degree of the other CDS nodes. So final CDS is found after pruning process covers nodes are 1, 2, 4, 8, 9,11and 12. This final CDS is minimum and known as MCDS

3.2. Algorithm proposed

In this project we are proposing an algorithm to find a routing in WSN. This algorithm consists of two phases. In phase I, a random network with n nodes which are randomly placed in specified area m x m is generated. In phase II by applying the algorithm proposed minimum connected dominating set if found, using which routing is achieved for the network

Phase I

A random network with n nodes which are randomly placed in specified area m X m is generated

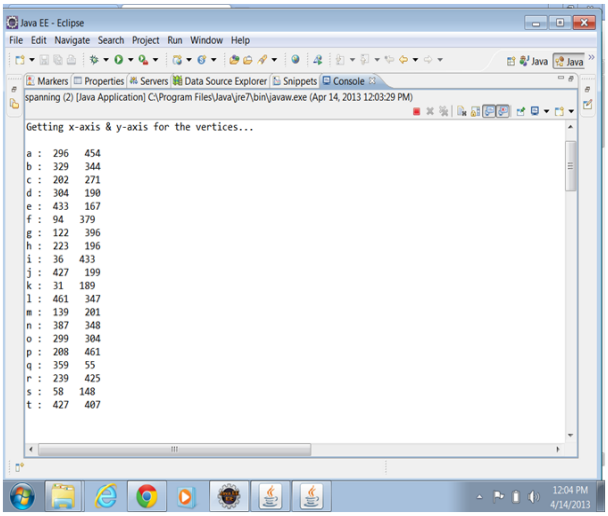
Phase II

By applying the algorithm proposed in [6] Minimum Connected Dominating Set is found, using which routing is achieved for the network.

Implementation

Random graphs were introduced by Erdos and Renyi in the late fifties. The random graph model generates a graph that has a number of nodes which are connected randomly by undirected edges. The algorithm is implemented and tested using JAVA. The sample outputs of the proposed algorithm are given here.

Fig 3.11. Position of the nodes.



Maximum degree of the nodes

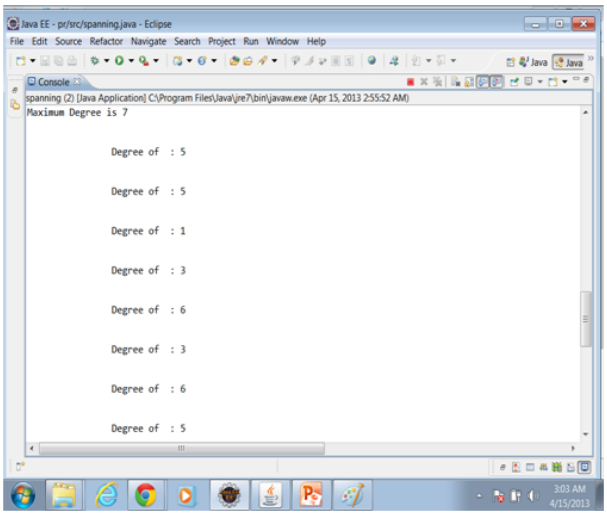
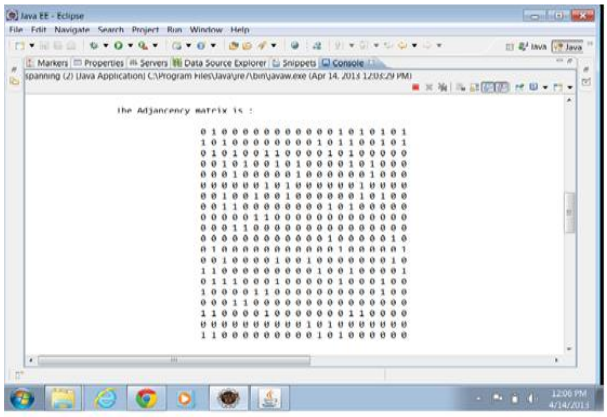
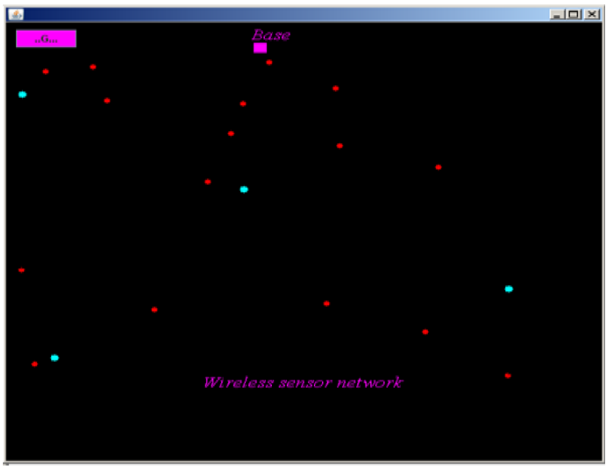
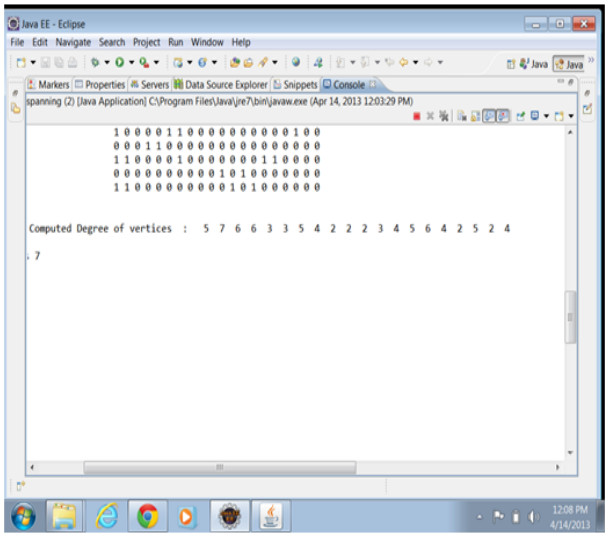


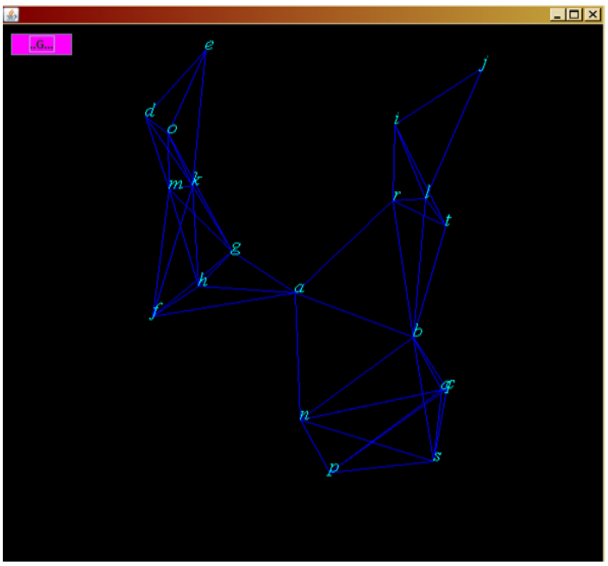
Fig 3.12: Adjacency Matrix of the Network.



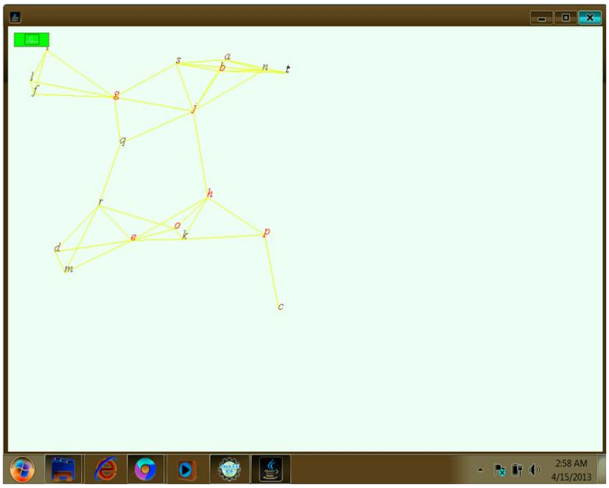


Degree of the nodes





Minimum Connected Dominating Set



4. CONCLUSION

The Minimum Connected Dominating Set (MCDS) act as virtual backbone in Wireless Sensor Networks. In this project work, an algorithm for finding MCDS using the concept of convex hull is studied. Another algorithm for finding power aware MCDS is also studied and implemented.

A new algorithm for finding a routing using MCDS in wireless sensor network is proposed. Proposed algorithm is implemented and tested using Java-Eclipse. The sample outputs are also included

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NEW SEPARATION AXIOMS VIA *GENERALIZED PRE OPEN SETS

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ABSTRACT

In this Paper, we introduce the notion of *g-p open sets and *g-p continuity in topological spaces. By utilizing these notions we introduce some weak separation axioms. Also we show that some basic properties of (*g, p)-T_i, (*g, p)-D_i spaces, for i = 0, 1, 2,...

Keywords: *g-p open, *g-p continuity, (*g, p)-T_i, (*g, p)-D_i.

1. INTRODUCTION

In 2000, Jafari introduced the notion of pre-regular p-open sets and further investigated its fundamental properties in (Jafari, 2006). The concept of preopen sets and precontinuous functions in topological spaces are introduced by A. S. Mashhour *et al* in 1982.

M.K.R.S Veerakumar introduced the notion of *g-p open sets which are weaker than open sets. Since then *g-open sets have been widely used in order to introduce new spaces and functions. In this paper X and Y denote the topological spaces. Let A be a subset of X. We denote the interior and the closure of a set A by Int(A) and Cl(A) respectively.

2. PRELIMINARIES

We recall the following definitions which are useful in the sequel.

2.1. Definition

A subset A of a space (X,τ) is called

i) a pre-open set (Mashhour *et al.,* 1982) if $A \subseteq$ int(cl(A))and a pre-closed set if cl(int(A)) $\subseteq$A.

ii) a semi-open set (Levine,1963) if A $\subseteq$cl(int(A)) and a semi-closed set if int(cl(A)) $\subseteq$ A.

iii) an α-open set (Njastad, 1965) if $A \subseteq$ int(cl(int(A))) and an α-closed set if cl(int(cl(A))) $\subseteq$ A.

iv) a semi-pre open set(Andrijevic,1986) if $A \subseteq$ cl(int(cl(A))) and a semi-pre closed set if int(cl(int(A))) $\subseteq$ A.

v) a regular open set (Stone,1937) if A = int(cl(A)) and a regular closed set if cl(int(A)) = A and

vi) δ–open set (Velicko, 1968) if for each $x \in A$ there exists a regular open set G such that $x \in G \subseteq A$.

The pre-closure (resp. semi-closure, α-closure, semi-preclosure) of a subset A of a space (X,τ) is the intersection of all pre-closed (resp. semi-closed, α-closed, semi-preclosed) sets that contain A and is denoted by pcl(A) (resp. scl(A), αcl(A), spcl(A)).

2.2. Definition

A subset A of a space (X,τ) is called a $\hat{g}$–closed set (Veera kumar, 2003) if cl(A) $\subseteq$ U whenever $A \subseteq U$ and U is semi-open in (X,τ).

Let (X,τ) be a space and let A be a subset of X. A is called *g-closed set (Veera kumar, 2006) if cl(A) $\subseteq$ U whenever $A \subseteq U$ and U is $\hat{g}$–open set of (X,τ). The complement of a *g-closed set is called *g-open. The intersection of all *g-closed (resp. δ-closed) sets containing A is called the *g-closure (resp. δ–closure) of A and is denoted by cl_{*g} (A) (resp. cl_{δ} (A)).

2.3. Definition

A subset A of a space (X,τ) is called a δ-preopen (Raychaudhuri and Mukherjee, 1993) if A int(cl_{δ} (A)). A family of δ-preopen sets in a topological space (X,τ) is denoted by $\delta PO(X,\tau)$.

3. *GENERALIZED PRE OPEN SETS

3.1. Definition

A subset A of a space (X,τ) is said to be *g-p-open if A int(cl_{*g} (A)). The complement of a *g-p-open sets is said to be *g-p-closed. The family of all *g-p-open (resp. *g-p-closed) sets in a topological space (X,τ) is denoted by $*gPO(X,\tau)$ (resp. $*gPC(X,\tau)$).

3.2. Definition

Let A be a subset of a topological space (X,τ). The intersection of all *g-p-closed (resp. δ-pre-closed) sets containing A is called the *g-p-closure (resp. δ–closure (Raychaudhuri and Mukherjee, 1993)) of A and it is denoted by pcl_{*g} (A) (resp. pcl_{δ} (A).

3.3. Definition

Let (X,τ) be a topological space. A subset U of X is called (*g,p) – neighbourbood of a point $x \in X$ if there exists a *g-p-open set V such that $x \in V \subseteq U$.

3.4. Theorem

For the *g-p-closure of subsets A, B in a topological space (X,τ), the following properties hold:

(i) A is *g-p-closed in (X,τ) if and only if $A = pcl_{*g}(A)$,

(ii) If $A \subset B$, then $pcl_{*g}(A) \subset pcl_{*g}(B)$

(iii) $pcl_{*g}(A)$ is *g-p-closed, that is $pcl_{*g}(A) = pcl_{*g}(pcl_{*g}(A))$ and

(iv) $x \in pcl_{*g}(A)$if and only if $A \cap V \neq \phi$ for every $V \in$ *gPO(X,τ) containing x.

Proof: It is obvious.

3.5. Theorem

For a family of subsets of a topological space (X,τ), the following properties hold:

(i) $pcl_{*g}(\cap\{A_\beta : \beta \in \Delta\}) \subset \cap\{pcl_{*g}(A_\beta) : \beta \in \Delta\}$

(ii) $pcl_{*g}(\cup\{A_\beta : \beta \in \Delta\}) \supset \cup\{pcl_{*g}(A_\beta) : \beta \in \Delta\}$

Proof:

(i) Since $\cap_{\beta \in \Delta} A_\beta \subset A_\beta$ for each $\beta \in \Delta$, by theorem 3.4, we have $pcl_{*g}(\cap_{\beta \in \Delta} A_\beta) \subset pcl_{*g}(A_\beta)$ for each $\beta \in \Delta$ and hence $pcl_{*g}(\cap_{\beta \in \Delta} A_\beta) \subset \cap_{\beta \in \Delta} pcl * gA_\beta$.

(ii) Since $A_\beta \subset \cup_{\beta \in \Delta} A_\beta$ for each $\beta \in \Delta$, by theorem 3.4, we have $pcl_{*g}(A_\beta) \subset pcl_{*g}(\cup_{\beta \in \Delta} A_\beta)$ for each $\beta \in \Delta$ and hence $\cup_{\beta \in \Delta} pcl * gA_\beta \subset pcl_{*g}(\cup_{\beta \in \Delta} A_\beta)$.

3.6. Theorem

Every *g-p-open sets is pre-open.

Proof: It follows from the definitions. The converse of the above theorem need not be true by the following example.

3.7. Example

Let X = {a, b, c} and τ = { X , ϕ , {a,b}}. Here {a,c} is not *g-p-open but however it is pre-open, since the 8g-p-open sets are X, ϕ, {a}, {b}, {a,b} and the pre-open sets are X, ϕ, {a}, {b}, {a,b}, {a,c}, {b,c}.

3.8. Theorem

(i) Every pre-open set is δ–pre-open (Caldas, 2010).

(ii) Every *g-p-open set is δ–pre-open.

Proof (ii): It follows from (i) and theorem 3.6.

3.9. Definition

A subset A of a topological space (X,τ) is called a $D_{(*g,p)}$ – set (resp. D_p – set , $D_{(\delta,p)}$ – set (Caldas, 2010)) if there are two U, V ∈ *gPO(X,τ) (resp. PO(X,τ), δPO(X,τ)) such that $U \neq X$ and $A = U - V$.

It is true that every *g-p-open (resp. pre-open) set U different from X is a $D_{(*g,p)}$ – set (resp. D_p – set) if $A = U$ and $V = \phi$.

3.10. Definition

A topological space (X,τ) is said to be

(1) (*g, p)-D_0 (resp. pre-D_0 (Caldas,2001; Jafari, 2001), (δ,p)- D_0 (Caldas, 2010)) if for any distinct pair of points x and y of X there exist a $D_{(*g,p)}$ – set (resp. D_p – set, $D_{(\delta,p)}$ – set) of X containing x but not y or a $D_{(*g,p)}$ – set (resp. D_p – set, $D_{(\delta,p)}$ – set) of X containing y but not x.

(2) (*g, p)-D_1 (resp. pre-D_1 (Caldas,2001; Jafari,2001), (δ,p)- D_1 (Caldas, 2010)) if for any distinct pair of points x and y of X there exist a $D_{(*g,p)}$ – set (resp. D_p – set, $D_{(\delta,p)}$ – set) of X containing x but not y or a $D_{(*g,p)}$ – set (resp. D_p – set, $D_{(\delta,p)}$ – set) of X containing y but not x.

(3) (*g, p)-D_2 (resp. pre-D_2 (Caldas,2001; Jafari, 2001), (δ,p)- D_2 (Caldas, 2010)) if for any distinct pair of points x and y of X there exists disjoint $D_{(*g,p)}$ – set (resp. D_p – set, $D_{(\delta,p)}$ – set) G and E of X containing x and y, respectively.

3.11. Definition

A topological space (X,τ) is said to be

(1) (*g, p)-T_0 (resp. pre-T_0 (Kar and Bhattacharyya, 1990; Nour, 1989) (δ,p)- T_0 (Caldas, 2005)) if for any distinct pair of points x and y of X there exist a *g-p-open (resp. pre-open, δ–pre-open) set U in X containing x but not y or a *g-p-open (resp. pre-open, δ–open) set V in X containing y but not x.

(2) (*g, p)-T_1 (resp. pre-T_1 (Kar and Bhattacharyya, 1990; Nour, 1989), (δ,p)- T_1 (Caldas, 2005)) if for any distinct pair of points x and y of X there exist a *g-p-open (resp. pre-open, δ–pre-open) set U in X containing x but not y and a *g-p-open (resp. pre-open, δ–pre-open) set V in X containing y but not x.

(3) (*g, p)-T_2 (resp. pre-T_2 (Kar and Bhattacharyya, 1990; Nour, 1989), (δ,p)- T_2 (Caldas, 2005)) if for any distinct pair of points x and y of X there exist a *g-p-open (resp. pre-open, δ–pre-open) sets U and V in X containing x and y, respectively, such that $U \cap V = \phi$.

3.12. Remark

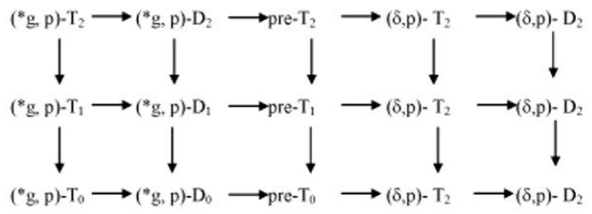
(i) If (X,τ) is (*g, p)-T_i, then it is (*g, p) – T_{i-1}, i = 1, 2.

(ii) If (X,τ) is (*g, p)-T_i, then it is (*g, p) – D_i, i = 0,1, 2.

(iii) If (X,τ) is (*g, p)-D_i, then it is (*g, p) – D_{i-1}, i = 1, 2.

(iv) If (X,τ) is (*g, p)-D_i, then it is pre–T_i, i = 0,1, 2.

By the above Remark 3.12 and [4], we have the following diagram.



3.13.Theorem

For a topological space (X,τ), the following properties hold: (X,τ) is (*g, p)-D_1 if and only if it is (*g, p)-D_2.

Proof:

Sufficiency Part: This follows from Remark 3.12.

Necessity Part: Suppose X is a (*g, p)-D_1. Then for each distinct pair x, y ∈ X, we have $D_{(*g,p)}$ – sets G_1 and G_2 such that x ∈ G_1, y ∉ G_1; y ∈ G_2, x ∉ G_2. Let $G_1 = U_1/ U_2$, $G_2 = U_3/ U_4$, where U_1, U_2, U_3, U_4 ∈ *gPO(X,τ). From x ∉ G_2 we have either x ∉ U_3 or x ∈ U_3 and x ∈ U_4. We discuss the two cases separately.

(1) x ∉ U_3. From y ∉ G_1 we have two subcases:

(a) y ∉ U_1. From x ∈ U_1/ U_2 we have x ∈ $U_1/ (U_2 \cup U_3)$ and from y ∈ U_3/ U_4 we have y ∈ $U_3/ (U_1 \cup U_4)$. It is easy to see that $(U_1/ (U_2 \cup U_3)) \cap (U_3/ (U_1 \cup U_4)) = \phi$.

(b) y ∈ U_1 and y ∈ U_2. We have x ∈ U_1/ U_2, y ∈ U_2 and $(U_1/ U_2) \cap U_2 = \phi$.

(2) x ∈ U_3 and x ∈ U_4. We have y ∈ U_3/ U_4, x ∈ U_4 and $(U_3/ U_4) \cap U_4 = \phi$.

From the discussion above we know that the space X is (*g, p)-D_2.

3.14. Definition.

A point x ∈ X which has only X as the (*g, p) – neighbourhood is called a (*g, p)- neat point.

3.15.Theorem

If a topological spaces (X,τ) is (*g, p)-D_1, so each point x of X is contained in a $D_{(*g,p)}$ – set O = U / V and thus in U. By definition U ≠ X. This implies that x is not a (*g, p)-neat point.

3.16. Definition

A topological space (X,τ) is (*g, p)-symmetric if x and y in X, x ∈ $pcl_{*g}(\{y\})$ implies y ∈ $pcl_{*g}(\{x\})$.

3.17. Theorem

For a topological space (X,τ), the following properties hold.

(1) If {x} is *g-p-closed for each x ∈ X, then (X,τ) is (*g, p)-T_1.

(2) Every (*g, p)-T_1 space is (*g, p)-symmetric.

Proof:

(1) Suppose {p} is *g-p-closed for every p ∈ X. Let x, y ∈ X with x ≠ y. Now x ≠ y implies y ∈ X / {x}. Hence X / {x} is a *g-p-open set contained in y but not containing x. Similarly X/{y} is a *g-p-open set contained in x but not containing y. Accordingly X is a (*g, p)-T_1 space.

(2) Suppose that y ∉ pcl_{*g} ({x}). Then, since x ≠ y, there exists a *g-p-open set U containing x such that y ∉ U and hence x ∉ pcl_{*g} ({y}). This shows that x ∈ pcl_{*g} ({y}) implies y ∈ pcl_{*g} ({x}). Therefore (X,τ) is (*g, p)-symmetric.

3.18. Definition

A function f: $(X,\tau) \to (Y, \sigma)$ is said to be *g-precontinuous if for each x ∈ X and each *g-p-open set V containing f(x), there is a *g-p-open set U in X containing x such that f(U) ⊆ V.

3.19. Theorem.

If f: $(X,\tau) \to (Y, \sigma)$ is a *g-precontinuous surjective function and E is a $D_{(*g, p)}$-set in Y, then the inverse image f^{-1}(E) is a $D_{(*g, p)}$-set in X.

Proof:

Let E be a $D_{(*g, p)}$-set in Y. Then there are *g-p-open sets U_1 and U_2 in Y such that $E = U_1/U_2$ and $U_1 \neq Y$. By the *g-precontinuity of f, $f^{-1}(U1)$ and $f^{-1}(U_2)$ are *g-p-open in X. Since $U_1 \neq Y$, we have $f^{-1}(U1) \neq X$. Hence $f^{-1}(E) = f^{-1}(U1) / f^{-1}(U_2)$ is a $D_{(*g, p)}$-set.

3.20. Theorem

If (Y, σ) is (*g, p)-D_1 and f: $(X,\tau) \to (Y, \sigma)$ is a *g-precontinuous bijection, then (X,τ) is (*g, p)-D_1.

Proof:

Suppose that Y is a (*g, p)-D_1 space. Let x and y be any pair of distinct points in X. Since F is injective and Y is (*g, p)-D_1, there exist $D_{(*g, p)}$-sets G_x and G_y of Y containing f(x) and f(y), respectively,

such that f(y) ∉ G_x and f(x) ∉ G_y. By theorem 3.19, $f^{-1}(G_x)$ and $f^{-1}(G_y)$ are $D_{(*g,\ p)}$-sets in X containing x and y, respectively, such that y ∉ $f^{-1}(G_x)$ and x ∉ $f^{-1}(G_y)$. This implies that X is a (*g, p)-D_1 space.

3.21. Theorem

A topological space (X,τ) is (*g, p)-D_1 if and only if for each pair of distinct points x, y ∈ X, there exists a *g-pre-continuous surjective function f: $(X,\tau) \to (Y, \sigma)$ such that f(x) and f(y) are distinct, where (Y, σ) is a (*g, p)-D_1 space.

Proof:

Necessity: For every pair of distinct points of X, it suffices to take the identity function on X.

Sufficiency: Let x and y be any pair of distinct points in X. By hypothesis there exists a *g-pre-continuous, surjective function f of a space X onto a (*g, p)-D_1 space Y such that f(x) ≠ f(y). By theorem 3.13, there exist disjoint $D_{(*g,\ p)}$-sets G_x and G_y in Y such that f(x) ∈ G_x and f(y) ∈ G_y. Since f is *g-pre-continuous and surjective, by theorem 3.20, $f^{-1}(G_x)$ and $f^{-1}(G_y)$ are disjoint $D_{(*g,\ p)}$-sets in X containing x and y, respectively, hence by theorem 3.13, X is a (*g, p)-D_1 space.

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ON b-mI-OPEN SETS AND b-mI- CONTINUOUS FUNCTIONS

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ABSTRACT

The purpose of this paper is to introduce b-mI-open sets in ideal minimal spaces and to investigate the relationships between minimal spaces and ideal minimal spaces. Furthermore, decomposition of continuous functions are established.

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Keywords : b-mI-open sets ,b-I-open set, b-mI-continuous functions.

1. INTRODUCTION

An ideal (Kuratowski, 1996) I on a nonempty set X is a nonempty collection of subsets of X which satisfies (i) A ∈ I and B ⊂ I and (ii) A ∈ I and B ∈ I implies A ∪ B ∈ I. Given a topological space (X, τ) with an ideal I on X and if P(X) is the set of all subsets of X, a set operator (.)*: P(X) → P(X), called a local function (6) for A with respect to τ and I is defined as follows: for A ⊂ X , A*(I , τ) = {x ∈ X : U ∩ A ∉ I for every U ∈ τ (x)} where τ (x) = { U ∈ τ : x ∈ U}. A Kuratowski closure operator cl*(.) for a topology τ*(I ,τ), called the τ - topology, finer than τ is defined by cl*(A) = A ⊂ A* (I , τ) (Vaidyanathaswamy, 1945). A subset A of an ideal space is said to be *-dense in itself (Hayashi,1986). (resp. *-closed (Jankovic and Hamlett, 1986)) if A ⊂ A* (resp.A* ⊂ A). By a space (X, τ), we always mean a topological space (X, τ) with no separation properties assumed. If A ⊂ X, cl(A) will, respectively, denote the closure and interior of A in (X, τ) and int*(A) will denote the interior of A in (X, τ). The notion of I-open sets was introduced by Jankovic *et al*. in 1992, further it was investigated by Abd El-Momsef. In 1965, Njastad initiated the investigation of α- open sets, Hatir and Noiri introduced the notion of α-I-open sets in an ideal topological spaces (X, τ,I), where τ is a topology and I is an ideal.

Maki *et al*. (1996) introduced the notion of minimal structure and minimal spaces as a generalization of topological spaces on a given nonempty set. Also, generalized topologies which are other generalization of topology were defined by Csaszar in 2002. Further, it was studied by Popa and Noiri in 2000. A subfamily $\mathcal{M}$ of the power set P(X) of a non empty set X a minimal structure, if ϕ, X ∈ $\mathcal{M}$. (X, $\mathcal{M}$) is called a minimal space (m-space). A subset A of X is said to be m-open (Maki, *et al .,* 1996) if A ∈ $\mathcal{M}$. The complement of a m-open set is called a m-closed set. Define m-int(A) = ∪{U : U ⊂ A,U ∈ $\mathcal{M}$} and m-c(A) = ∩{F : A⊂F, X-F∈ $\mathcal{M}$}. A minimal (X, $\mathcal{M}$) has the property[$\mathcal{U}$] (Popa and Noiri, 2000) if the arbitrary union of m-open sets is again a m-open set.

Ozbakir and Yildirim in 2009 have defined the minimal local function A_m^* in an ideal minimal space (X, $\mathcal{M}$, I). The notion of α-mI-open set, semi-mI-open set, β-mI-open set in (X, $\mathcal{M}$, I) were introduced and investigated by Parimala. In this paper, by using the local function A_m^* we introduce and investigate the notion of α-mI-open set in (X, $\mathcal{M}$, I). Furthermore, decompositions of continuous function are established.

2. PRELIMINARIES

2.1. Definition (Ozbakiri and Yildirim, 2009) Let (X, $\mathcal{M}$) be a minimal space with an ideal I and X (.)*m be a set operator from P(X) to P(X) (P(X) is the set of all subsets of X). For a subset A⊂X, A_m^*(I, $\mathcal{M}$) ={ x ∈ X: U_m∩ A ∉I; for every U_m ∈ U_m (x)} is called the minimal local function of A with respect to I and $\mathcal{M}$. We will simply write A_m^* for A_m^*(I, $\mathcal{M}$).

2.2. (*Theorem* Ozbakiri and Yildirim, 2009) Let (X, $\mathcal{M}$) be a minimal space with I,I′ ideals on X and A, B be subsets of X. Then

(i) A⊂B ⇒ $A_m^* \subset B_m^*$,

(ii) I⊂I′⇒ $A_m^*(I') \subset A_m^*(I)$,

(iii) A_m^*=m-cl(A_m^*) ⊂m-cl(A),

(iv) $A_m^* \cup B_m^* \subset (A \cup B)_m^*$

(v) $(A_m^*)_m^* \subset A_m^*$

2.3. Remark (Ozbakiri and Yildirim, 2009) If (X, $\mathcal{M}$) has property (Lashien and Nasef, 1992) then $A_m^* \cup B_m^* = (A \cup B)_m^*$

Definition 2.4. (Ozbakiri and Yildirim, 2009) Let (X, $\mathcal{M}$) be a minimal space with an ideal I on X. The set operator m-cl* is called a minimal *-closure and

is defined as m-cl*(A) = A∪ A_m^* for A⊂X. We will denote by $\mathcal{M}^*(I,\mathcal{M})$ the minimal structure generated by m-cl*, that is, $\mathcal{M}^*(I,\mathcal{M})$={ U⊂X: m-cl*(X-U)=X-U}. $\mathcal{M}^*(I,\mathcal{M})$ is called *-minimal structure which is finer than $\mathcal{M}$. The elements of $\mathcal{M}^*(I,\mathcal{M})$ are called minimal *-open (briefly, m*-open) and the complement of an m*-open set is called minimal *-closed (briefly, m*-closed).

Throughout the paper we simply write $\mathcal{M}^*$ for $\mathcal{M}^*(I,\mathcal{M})$. If I is an ideal on X, then $(X,\mathcal{M},I)$ is called an ideal minimal space.

2.5.Proposition (Ozbakiri and Yildirim, 2009) The set operator m-cl* satisfies the following conditions:

(i) A⊂m-cl*(A),

(ii) m-cl*(ϕ)= ϕ and m-cl*(X)=X,

(iii) If A⊂B, then m-cl*(A) ⊂ m-cl*(B),

(iv) m-cl*(A)∪ m-cl(B) ⊂ m-cl*(A∪B).

2.6.Remark

If $(X,\mathcal{M})$ has property (Lashien and Nasef, 1992) then m-cl*(m-cl*(A))=m-cl*(A) and m-cl*(A)∪ m-cl*(B)= m-cl*(A∪B).

2.7.Lemma (Renukadevi, *et al.,* 2005) Let (X,τ,I) be an ideal space and A⊂ X. If A⊂A*, then A* = cl(A*) = cl(A) = cl*(A).

2.8.Definition

A subset A of an ideal minimal space $(X,\mathcal{M},I)$ is said to be

(i) α-mI-open set (Parimala, 2010) if A⊂ m-int(m-cl*(m-int(A))).

(ii) semi-mI-open set (Parimala, 2010) if A⊂ m-cl*(m-int(A))).

(iii) β-mI-open set (Parimala, 2010) if A⊂ m-cl(m-int(m-cl*(A))).

(iv) mI-open set (Ozbakiri and Yildirim, 2009) if A⊂ m-int(A_m^*)

(v) pre-mI-open set (Parimala, 2010) if A⊂m-int(m-cl*(A)).

3. B-MI-OPEN SET AND B-MI-CLOSED SET

3.1.Definition

A subset A of an ideal minimal space $(X,\mathcal{M},I)$ is said to be a b-mI-open set if A⊂m-cl(m-int(A)) ∪ m-int(m-cl(A)). The complement of a b-mI-open set is a b-mi-closed set.

3.2. Theorem.

For a subset of an ideal minimal space, the following condition hold.

(i) Every b-mI-open set is b-m-open.

(ii) SmIO(X,$\mathcal{M}$) ∪ PmIO(X,$\mathcal{M}$) ⊂ BmIO(X,$\mathcal{M}$).

(iii) Every m-open set is b-mI-open.

Proof. (i) Let A be b-mI-open set. Then we have

A⊂m-int(m-cl*(A)) ∪ cl*(m-int(A))

⊂m-int(A_m^* ∪ A) ∪((m-int(A))* ∪(m-int(A)))

⊂m-int(m-cl(A) ∪ A) ∪(m-cl(m-int(A)) ∪(m-int(A)))

⊂m-int(m-cl(A) ∪m-cl(m-int(A))

Therefore this shows that A is b-m-open.

The proof is obvious for (ii),(iii).

3.3.Theorem

For a subset of an ideal minimal space, the following conditions hold.

(i) Every pre-mI-open set is b-mI-open.

(ii) Every semi-mI-open set is b-mI-open.

(iii) Every b-mI-open set is β–mI-open.

Proof. The proof is obvious for (i), (ii).

(iii) Let A be an b-mI-open set. Then we have

A⊂m-int(m-cl*(A)) ∪cl*(m-int(A))

⊂m-cl(m-int(m-cl*(A))) ∪[(m-int(A))* ∪m-int(A)]

⊂m-cl(m-int(m-cl*(A))) ∪(m-cl(m-int(A)) ∪m-int(A))

⊂m-cl(m-int(m-cl*(A))) ∪(m-cl(m-int(A)))

⊂m-cl(m-int(m-cl*(A)))

Therefore this shows that A is an β–mI-open.

3.4. Example

(i) Let X={a,b,c,d}, $\mathcal{M}$={X ϕ,{a,b},{b,c},{c,d}} and I= ϕ. Let A={a,b,c} is b-mI-open but not semi-mI-open set.

(ii) Let X={a,b,c,d}, $\mathcal{M}$={X ϕ,{a},{b},{a,b,c},{b,c},{a,c}} and I={{a}, ϕ}. Let A={a,c,d} is β–mI-open but not pre-mI-open set.

(iii) Let X={a,b,c,d}, $\mathcal{M}$={X ϕ,{a},{b},{b,c,d}} and I={ ϕ , {b},{c},{b,c}}. Let A={a,b,c} is β–mI-open but not b-mI-open set.

3.5. Theorem

Let A be a b-mI-open set such that int(A)= ϕ, then A is pre-mI-open.

Proof. Since A⊂m-int(m-cl*(A)) ∪cl*(m-int(A))=m-int(m-cl*(A)) ∪m-cl*(ϕ)=m-int(m-(cl*(A))). Then A is pre-mI-open.

3.6.Theorem

Let $(X,\tau,\mathcal{M})$ be an ideal minimal space and A,B subset of X.

(i) If $U_\alpha \in BmIO(X,\tau)$ for each $\alpha\in\Delta$, then $\cup\{U_\alpha: \alpha\in\Delta\} \in BmIO(X,\tau)$.

(ii) If $A_\alpha\in BmIO(X,\tau)$ and $B\in\mathcal{M}$, then $A\cap B \in BmIO(X,\tau)$

Proof. (i)Since $U_\alpha \in BmIO(X,\tau)$, we have $U_\alpha\subset$m-int(m-cl*(U_α)) $\cup$ cl*(m-int(U_α)) for every $\alpha\in\Delta$.

$\subset\cup_\alpha\in_\Delta[\{$(m-int($U_\alpha$))* $\cup$m-int(U_α)$\}\cup$(m-int((${U_\alpha^*}\cup U_\alpha$))]

$\subset[\{$(m-int($\cup_\alpha\in_\Delta U_\alpha$))* $\cup$m-int($\cup_\alpha\in_\Delta U_\alpha$)$\}\cup$(m-int(($\cup\alpha\in\Delta {U_\alpha^*}\cup(\cup_\alpha\in_\Delta U\alpha)$))]

$[cl*(m-int(\cup_\alpha\in_\Delta U\alpha))\cup$m-int(m-cl*($\cup_\alpha\in_\Delta U\alpha$))] Therefore, $\cup_\alpha\in_\Delta U\alpha$ is b-mI-open.

(ii) Let $A\in BmIO(X,\mathcal{M})$ and $B\in\mathcal{M}$.Then $A\subset$m-int(m-cl*(A)) $\cup$cl*(m-int(A))

$A\cap B \subset$[m-int(m-cl*(A)) $\cup\ cl*(m-int(A))]\cap B$

$\subset[\{$m-int(A))* $\cup$m-int(A)$\}\cup$(m-int(A*$\cup$A))] $\cap B$

$\subset[\{$m-int(A$\cap B$))* $\cup$m-int(A$\cap$B)$\}\cup$(m-int((A$\cap B$)* $\cup$(A$\cap B$)))]

$\subset$m-int(m-cl*(A$\cap B$)) $\cup$cl*(m-int(A$\cap B$)). Then A$\cap B$ is b-mI-open.

(i.e.) A$\cap B$ BmIO(X,τ).

3.7. Definition

A subset A of a space $(X,\mathcal{M},I)$ is said to be a b-mI-closed set if its complement is b-mI-open.

3.8. Theorem

If a subset A of a space $(X,\tau,\mathcal{M})$ is b-mI-closed then m-int(m-cl*(A)) $\cap$m-cl*m-int(A)) $\subset$A.

Proof. Since A is b-mI-closed, X-A BmIO(X,$\mathcal{M}$) and since $\mathcal{M}$* is finer than $\mathcal{M}$ X-A$\subset cl*(m-int(X-A)\cup$ m-int(m-cl*(X-A) $\cup\ m-int(m-cl*(X-A)))$

$\subset$cl(m-int(X-A)) $\cup$m-int(m-cl(X-A)))

=[X-cl(m-int(A)] $\cup$[X-(m-int(m-cl(A)))]

=X-[cl(m-int(A) $\cap$(m-int(m-cl(A)))]. Therefore, m-int(m0cl*(A)) $\cap$m-cl*(m-int(A)) $\subset$A.

3.9. Corollary

Let A be a subset of $(X,\tau,\mathcal{M})$ such that X-[m-int(m-cl*(A))]=m-cl*(m-int(X-A)) and X-[m-cl*m-int(A))]=m-int(m-cl*(X-A)). Then A is b-mI-closed if and only if m-int(m-cl*(A)) $\cap$m-cl*(m-int(A)) $\subset$A.

Proof. Necessity:

This is an immediate consequence of Theorem 3.8.

Sufficiency:

Let m-int(m-cl*(A)) $\cap$m-cl*(m-int(A)) $\subset$A. Then X-A$\subset$X-[cl(m-int(A)] $\cap$(m-int(m-cl(A)))] $\subset$[X-m-cl*(m-int(A))] $\cup$[X-m-int(m-cl*(A))]=[cl*(m-int(X-A) $\cup$m-int(m-cl*(X-A))). Thus X-A is b-mI-open and so A is b-mI-closed.

4. DECOMPOSITION OF CONTINUITY VIA MINIMAL IDEALS

4.1.Definition

A function f: $(X,\mathcal{M},I)\to(Y,\sigma)$ is said to be b-mI-continuous if for every V$\in\sigma$,f^{-1}(V) is an b-mI-open set of $(X,\mathcal{M},I)$.

4.2.Definition

A function f: $(X,\mathcal{M})\to(Y,\sigma)$ is said to be b-m-continuous if for every V$\in\sigma$,f^{-1}(V) is an b-m-open set of $(X,\mathcal{M})$.

4.3.Theorem

If a function f: $(X,\mathcal{M},I)\to(Y,\sigma)$ is said to be b-mI-continuous then f is b-m-continuous.

Proof. The proof is obvious.

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DYNAMIC PRICING MODEL USING PRICE MULTIPLIERS FOR ONLINE BUS TICKET BOOKING

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ABSTRACT

Dynamic pricing is a price determination by the buyer and seller at the time of transaction. In the present study we propose a dynamic pricing approach for the online bus ticket booking. The proposed approach is based on having "price multipliers" that vary around "1" and provide a varying discount/premium over the base price. The price multipliers are functions of certain influencing variables .The values of price multipliers are determined based on analysis of price multipliers and its demand variation. We have tested the proposed approach by successfully applying it to the online bus ticket booking for the route "Chennai - Mudalur 2012" of Ticketgoose.com, an online bus ticket booking firm as a case study.

Keywords: Dynamic pricing, Price multiplier, influencing variables

1. INTRODUCTION TO DYNAMIC PRICING

Dynamic pricing is a popular method of revenue management, especially when a firm needs to sell a given stock by deadline. Revenue management is a scientific method that helps firms to improve profitability of their business. The goal of dynamic pricing is to increase the revenue by discriminating customers who arrive at different times. For instance, if a firm faces a high level of demand, it has an incentive to increase the price to reserve some products for later customers who may be willing to pay more. On the other hand, if the demand is low the firm wishes to lower the price in order to induce the demand, because the product has no value (or very low value) after the deadline. Therefore, the retailer can dynamically adjust the price between any of a finite number of allowable prices (Richard Chatwin, 2000).

Dynamic pricing strategy often discounts excessive items, and charges a higher price for scarce items. A firm can often increase its revenue by carefully adjusting its product price over time.

The industry which is most commonly mentioned in terms of its adopting dynamic pricing strategies is airline transportation. Similarly, items such as hotel rooms, sports tickets, and seasonal fashion goods that become worthless if they are not sold by a specific time are all suited to dynamic pricing because these perishable items must be sold prior to the time at which they are unsalable. In all of these cases, the sellers can improve their revenue by dynamically adjusting the price of the perishable products rather than adopting a fixed price (Ue-Pyng Wenand Yen-Hsiang Chen, 2005).

By employing dynamic pricing, the act of changing prices over time within a marketplace, sellers have the potential to increase their revenue by selling goods to buyers "at the right time, at the right price."

1.1. Revenue management

Background

In the early 1970s, some airlines began offering restricted discount fare products that mixed discount and higher fare passengers in the same aircraft compartments. For example, BOAC (now British Airways) offered early bird bookings that charged lower fares to passengers who booked at least twenty- one days in advance of flight departure.

This innovation offered the airline the potential of gaining revenue from seats that would otherwise fly empty; however, it presented them with the problem of determining the number of seats that should be protected for late booking, full fare passengers. If too few seats were protected, the airline would spill full fare passengers; if too many were protected, flights would depart with empty seats.

No simple rule, like protecting a fixed percentage of capacity, could be applied across all flights because passenger booking behaviour varied widely with relative fares, itineraries, season, day of week, time of day, and other factors. It was evident that effective control of discount seats would require detailed tracking of booking histories, expansion of information system capabilities, and careful research and development of seat inventory control rules.

LITTLEWOOD (1972) of BOAC proposed that discount fare bookings should be accepted as long as their revenue value exceeded the expected revenue of future full fare bookings. This simple, two fare, seat inventory control rule (henceforth, Little

wood's rule) marked the beginning of what came to be called yield management and, later, revenue management.

1.2. Static and dynamic pricing

Pricing is major area of airline revenue management. Pricing is generally categorized into two parts static and dynamic.

In the static pricing, the price is set at being of the booking period. In the dynamic pricing, the price changes throughout the booking period.

There is a trend in pricing that promises to significantly change the way goods are marketed and sold. Sellers now offer special deals, tailored for individual customers, and are beginning to compute the right price to the right customer at the right time. This change has been largely due to the wiring of the economy through the internet, corporate networks, and wireless networks. Buyers are now able to quickly and easily compare products and prices, putting them in a better bargaining position. At the same time, the technology is allowing sellers to collect detailed data about customers' buying habits, preferences, even spending limits, so they can customize their products and prices (Narahari, 2005).

In the past, there was a significant cost associated with changing prices, known as the menu cost. For a company with a large product line, it could take months for price adjustments to filter down to distributors, retailers, and salespeople. Emergence of network technology has reduced menu cost and time to near zero. As buyers and sellers interact in the electronic world, the resulting dynamic prices more closely reflect the true market value of the products and services being traded (Narahari, 2005).

1.3. Dynamic pricing strategies

Segmented Pricing refers to the customers' willingness to pay more for a given service or product. For example, a product may be sold at one price with a warranty and a lower price without a warranty.

Pricing based on peak times is *peak user pricing*. For example, airlines companies often charge a higher price to travel during rush hour on Monday through Friday than at other times and on weekends.

Service time is another dynamic pricing strategy is to charge more for faster service. For example, same-day dry cleaning would cost more than overnight cleaning.

Some dynamic pricing strategies offer customers different prices based on when they buy which are termed as *time-based pricing*. For example, the airline may try to fill seats by lowering the price as the day of the flight draws closer.

Based on the *changing condition* sellers can maximize profits by lowering prices as sales fall, then raising prices again as demand increases.

1.4. Models used in dynamic pricing

A variety of mathematical models have been used in computing dynamic prices. Most ofthese models formulate the dynamic pricing problem as an optimization problem. Dependingon the specific mathematical tool used and emphasized, we provide a list of five categories of models (Narahari, 2005).

Inventory-based models: These are models where pricing decisions are primarily based on inventory levels and customer service levels.

Data-driven models: These models use statistical or similar techniques for utilizing data available about customer preferences and buying patterns to compute optimal dynamic prices.

Game theory models: In a multi-seller scenario, the sellers may compete for the same pool of customers and this induces a dynamic pricing game among the sellers. Game theoretic models lead to interesting ways of computing optimal dynamic prices in such situations.

Machine learning models: An e-business market provides a rich playground for online learning by buyers and sellers. Sellers can potentially learn buyer preferences and buying patterns and use algorithms to dynamically price their offerings so as to maximize revenues or profits.

Simulation models: It is well known that simulation can always be used in any decision making problem. A simulation model for dynamic pricing may use any of the above four models stated above or use a prototype system or any other way of mimicking the dynamics of the system.

1.5. Applications of dynamic pricing

Dynamic pricing practices appear in industries where it is impossible (or very costly) to increase the inventory level and there exists a deadline to sell the products. These industries include fashion goods, airlines, hotel rooms, rental cars, and highway congestion control.

The vendor of fashion apparel, which perishes when the season ends, has to set its product price during the season. The consumers of the

fashion goods enjoy more if they have the item in the earlier points of the season. For example, an air conditioner is more valuable at the beginning of the summer, so the consumers are willing to pay more in price earlier in summer. Using this fact, a retailer can potentially increase the revenue by charging a higher price at the beginning of the season and a lower price at the end of the season.

Airline industries often sell the airline tickets at a lower price to those consumers who buy their tickets early in advance, in order to reduce the risk of the airplane taking off with many vacant seats. If too many seats are sold at a low price, the airlines often would raise its ticket price, not only because the risk of having vacant seats is low but also because the airline wants to reserve seats for potential last-minute travellers.

The tourism industry is also one that makes frequent use of dynamic pricing. Different locations experience larger volumes of tourists at different times of the year. A beach resort, for instance, will experience much greater demand when it is summer in that location than it will during the winter, or if it is warm year-round at the beach resort, business might pick up when it is winter in other locations. Using dynamic pricing, a hotel will raise its prices during its peak season and lower them during the off-season.

Auction sites such as Ebay.com and Onsale.com have been successfully running auctions where people participate outbidding one another to purchase computers, electronics, sports equipment, etc. at dynamic prices that are governed by supply-demand characteristics.

1.6. Merits and demerits of dynamic pricing

Online dynamic pricing can help businesses in a variety of ways. It creates efficient markets by providing a mechanism to eliminate imperfect information, by increasing the geographic reach of smaller suppliers, and by streamlining purchasing processes.

By eliminating inefficiencies, suppliers are able to decrease costs and increase revenues on inventory, decrease overhead, eliminate costly middlemen, increase inventory turns, and create new.

In addition, dynamic pricing allows for "test pricing," and can yield increased revenue from new and unique items. All this is brought about through communities of buyers and sellers coming together to aggregate supply and demand. Internet Exchanges and auction technologies are creating a new class of intermediaries. These players create their own markets by inserting themselves into niches that previously had been dominated by entities offering only fixed-rate pricing, and offer advantages to both buyers and sellers in the process. The speed with which new companies can enter this space is increasing with the rollout of new auction applications that are faster and easier to implement.

Product pricing is one of the most important aspects of marketing that directly influences a business's ability to make profit and succeed. If a company sets prices too high, customers might choose to buy products elsewhere, while low prices may lead to a suboptimal amount of income. Dynamic pricing is a pricing method where businesses adjust prices based on consumer demand in an attempt to boost sales, which can potentially lead to several negative consequences.

2. PRICING PRACTICES PREVALENT IN THE MARKET TODAY

2.1. *Base price*

Base prices of the bus tickets are defined today based on the following factors:

- Travel distance
- Make of the bus
- Super luxury class - Includes Volvo, Mercedes and other top end buses
- Luxury class – Buses custom built on chassis of Tata/Leyland/Kinglongetc
- Climate control – A/C or Non A/C
- Seat Type – Push back, Semi sleeper (Push back seat with leg support), Sleeper (berths)
- Seat Layout – Number of seats in a row: 1+2, 2+2 etc
- Seat Position – Normally done for sleeper class buses. Lower berths are more comfortable than upper berths and hence many operators price them higher. Very rarely, a few operators price the rear seats lower than the front seats.

2.2. *Current practice of dynamic pricing*

Current practice of dynamic pricing is totally manual and might vary on both sides of the base price based on demand.

2.2.1. Non peak days (supply>demand)

Many operators lower their rates at boarding points and try to fill the buses. Lowering of rates is not done in their online sites or with the inventory available with aggregators.

Operators plying more than one bus in the same route consolidate their seats and ply lesser

number of services. Single bus operators may cancel their service and consolidate their tickets with another operator plying in the same route or sometimes just refund the money to the customer.

Consolidation and cancelling of services depends on two conditions – The amount of cargo that is booked on their bus and the number of bookings that have already happened on the return trip.

2.2.2. Peak days (demand>supply)

Many operators hike their fares based on their experience. Regular operators do not hike fares beyond a certain limit. Regular bus operators deploy all their spare buses on these dates at higher rates.

Special Bus operators (operators who lease buses and operate only on peak days) ply many services at very high rates depending on demand.

Special bus rates tend to be on the higher side due to the following factors:

- Demand is high and customers willing to buy tickets at a higher price
- Need for taking temporary permits for operating on specific days alone.
- On peak days, buses normally ply full only one way, i.e. from major metros to other destinations on the start of a holiday and in the reverse direction when the holidays or weekends is over. Operators will have to either ply the return trips empty or halt their buses in the destinations for the holiday period.

Few operators do not hike their fares even on peak days. Fare hike is based on the operators' previous experience. In recent days, we have seen many special bus operators quoting exorbitant rates only to revise them to a more reasonable level once they find that there are no takers at that price.

3. DYNAMIC PRICING MODELUSING PRICE MULTIPLIERS

The proposed dynamic pricing approach is based on having "price multipliers" and this idea was already validated for hotel revenue management problem by (Abd El-Moniem Bayoumi, 2001). These price multipliers provide a varying discount / premium over the base price. The base price is typically set by the bus operators.

Moreover, we have four multipliers that represent the "control variables". They will be multiplied by the base price, to obtain the final price. They vary around the value of 1, where a value that is lower than one corresponds to a discount with respect to the base price (for example 0.9 means the price is 10 % lower). Conversely, a value that is higher than 1 represents a premium over the base price.

The price multiplier values are determined based on analysis of price multipliers which affects the pricing decision. The advantage of this formulation is that it will give the bus operator a suggested price that has some relation with the price that he has determined during his experience. So, he can relate to the new price by observing its discount/premium in relation to his base price.

Each of the four multipliers corresponds to a variable that is known to have an influencing effect on pricing decisions. Specifically, the four variables that we selected are:

1. Demand over departure time

2. Demand over months

3. Booking rate variation over lead days (no. of days booked in advance)

4. Filled rate for a bus over a period of time

By determining the values of price multipliers final price can be calculated as shown in (1). Upper and lower limits for the price multipliers are set by the bus operators. By analysing demand over time, premium or discount over base price is applied.

The final price is given by the product of the reference price and the multipliers, as follows.

Final price =Base price*Departure time multiplier*Load multiplier*

Lead days multiplier*Filled rate multiplier (1)

The resulting price will reflect the discounts/premiums resulting from the different values of the influencing variables.

3.1. Price multipliers

The following are the four multipliers that determine the final price:

3.1.1. Departure time multiplier

Departure time multiplier corresponds to the price influence of bus tickets based on the demand variation over different departure times available for the selected route. Each departure time for the selected route shows different demand and therefore discount can be applied for the price multiplier when the demand is low and premium can be applied for the price multiplier when the demand is high. i.e., Firm decreases the ticket price when demand is low and increases when demand is high.

3.1.2. Load multiplier

Load multiplier corresponds to a price influencing variable which is based on the demand variation over seasons. Usually, holidays and summer seasons have high booking proportion. For e.g. April to May is a holiday season and therefore has high demand. So, firm increases the ticket price for high demand seasons and decreases price for lower demand seasons.

3.1.3. Lead days multiplier

Lead days are nothing but the number of days booked in advance before the departure date of a bus. Lead days multiplier corresponds to the price influence of ticket price based on the booking proportion over lead days. As per the analysis, booking proportion increases from seven days before the departure date.

Demand is very high on the day of travelling and low when booking starts from 20 days earlier before the departure date. Firm increases ticket price for customers booking in 0 – 7 days before departure date.

3.1.4. Filled rate multiplier

Filled rate multiplier corresponds to the price influence based on filled proportion for a trip. Demand is high when the bus is at most filled (>90%).During the high demand seasons buses have quick filling of seats. Therefore, Price is increased when filled rate is high and decreased when filled rate is low.

4. DYNAMIC PRICING FOR ONLINE BUS TICKET BOOKING

Dynamic pricing for online bus ticket booking is calculated using price multipliers: Departure time multiplier, Load multiplier, Lead days multiplier and Filled rate multiplier. Price multiplier values are determined from the analysis of price multipliers over demand and final price is obtained from the result (1).Demand corresponding to these price influencing factors varies according to the selected routes.

4.1. Organisation profile

The luxury bus industry today is worth 20,000 crores all over India. The four southern states will be contributing to around 5,000 crores to this market. Bus ticket pricing in the industry today is done manually by bus operators taking into consideration multiple factors and varies based on demand. Ticketgoose.com aims at devising a dynamic pricing mechanism which will allow operators define their prices dynamically taking into consideration the market factors.

About Ticketgoose.com

Ticket goose is promoted by Ticketgoose.com India Pvt. Ltd. and was launched in August 2007 is a ticketing web portal to provide online services to everyone travelling by bus. TicketGoose.com provides information like price, availability and booking facility for buses to over 5000 destinations and currently has 500+ bus operators as partners.

Ticket goose.com**'s** biggest advantage is its network of 3000+ channel partners who serve the customers without internet access. It is also a major supplier of inventory to other aggregation sites like Make My Trip, ixigo etc. Ticket goose. Com has been the torchbearer for organizing the hitherto unorganized bus industry in the country, by IT enabling operators with its unique and cost effective solutions. The Team at Ticketgoose.com, headed by Arun Athiappan, Co-Founder and CEO, comprises of highly qualified and dedicated technical professionals who are focused on ensuring hassle-free, cost effective and happy travel for all consumers who use its service.

4.2. Case study

Dynamic pricing is successfully tested for the route “CHENNAI– MUDALUR 2012”. The data points were collected from Vahana operating network ofTicketgoose.com. Vahana operating network is one which includes own operators of Ticket goose.

4.2. Route specifications

Mudalur is located in Thoothukudi district. Travelling duration is around 11 hrs and travelling distance is 654 kms. This route is a non-commercial route.

4.3. Results from previous analysis

For the selected route, demand over available departure times, season, lead days and Filled rate was analysed with the booking transactions for the year 2012 and the results are as follows:

Departure Time Multiplier

The Departure times available for the route are 16:00 hrs, 18:30 hrs, 19:15 hrs and 20:00 hrs. According to the analysis, demand is high for 19:15 hrs and low for 16:00 hrs. Premium over base price is given when demand is high and discount over base price is given when demand is low. As the selected

route is a non-commercial route people wish the trip that would not affect their regular routines. Travelling duration is around 11 hrs, so trip started at 19.15 hrs will arrive at the destination by 6.15 hrs. 18.30 hrs is having moderate demand.

Load Multiplier

Demand over seasons was analysed. According to the analysis, demand is high for May and June month and therefore premium over base price is given. Demand is low for November and December month and therefore discount over base price is given. Other seasons are having moderate demand.

Lead Days Multiplier

Booking rate is highly recorded on 7 days before departure date and very high on the day of travelling. Booking rate is very low for above 15 days booked in advance .Therefore, demand is very high on the day of travelling and so premium over base price is given. Discount over base price is given for lesser demand lead days.

Filled Rate Multiplier

Filled rate is higher on holiday and summer season and low on lesser demand conditions (for e.g. 15 days before departure, less preferred season).Premium over base price is given when filled rate is above 90% and discount over base price is given when bus is filled less than half.

4.4. Dynamic pricing

From the previous analysis and using the result (1) dynamic price for "Chennai-Mudalur" is calculated for higher, moderate and lower level of demands.

4.4.1. Determining price multipliers

Price multipliers vary around "1" where value lower than 1 corresponds to the discount over base price whereas value higher than 1 corresponds to the premium over base price.

Table 1. Price Multiplier values

Demand level	Price multiplier values
High	1.05 – 1.1 (5 – 10% premium)
Moderate	0.96 – 1.04 (1 – 4% discount or 1 – 4% premium)
Low	0.9 – 0.95 (5 – 10% discount)

Below are the price multiplier values determined from the analysis result.

Table 2. Price Multiplier values for Load Multiplier

Season	Demand level	Price multiplier values
May, June, August	High	1.05 – 1.1 (5 – 10% premium)
January,April, July	Moderate	0.96 – 1.04 (1 – 4% discount or 1 – 4% premium)
November, December, February, March, September, October	Low	0.9 – 0.95 (5 – 10% discount)

Table 3. Price Multiplier values for Departure Time Multiplier

Departure time	Demand level	Price multiplier values
19.15	High	1.05 – 1.1 (5 – 10% premium)
18.30	Moderate	0.96 – 1.04 (1 – 4% discount or 1 – 4% premium)
16	Low	0.9 – 0.95 (5 – 10% discount)

Table 4. Price Multiplier values for Lead days Multiplier

Lead days	Demand level	Price multiplier values
0 - 7	High	1.05 – 1.1 (5 – 10% premium)
7 - 15	Moderate	0.96 – 1.04 (1 – 4% discount or 1 – 4% premium)
15 - 60	Low	0.9 – 0.95 (5 – 10% discount)

Table 5. Price Multiplier values for Filled rate Multiplier

Filled rate	Demand level	Price multiplier values
70 – 97 %	High	1.05 – 1.1 (5 – 10% premium)
30 – 70 %	Moderate	0.96 – 1.04 (1 – 4% discount or 1 – 4% premium)
5 – 30 %	Low	0.9 – 0.95 (5 – 10% discount)

4.4.2. Calculating dynamic price

Final price is obtained as dynamic price which is the product of base price and price multipliers. Dynamic price for the selected route is calculated through MATLAB.

Implementation Metho

Price influencing booking details is taken as input. According to the inputs the demand level is obtained and final price is calculated with price multiplier values specified in the Tables 2, 3, 4 & 5.

In this method for the given level of demand, set of possible dynamic prices are calculated. Total price for the trip is also calculated automatically which gives convenience for the operators about selecting a dynamic price. Operator can select a dynamic price from a set of dynamic prices displayed and can be used for comparing with known revenue of the previous trip in which operator sold all tickets with different price.

Fig 1. Calculating possible dynamic prices for high demand (for 1 day booked in advance)

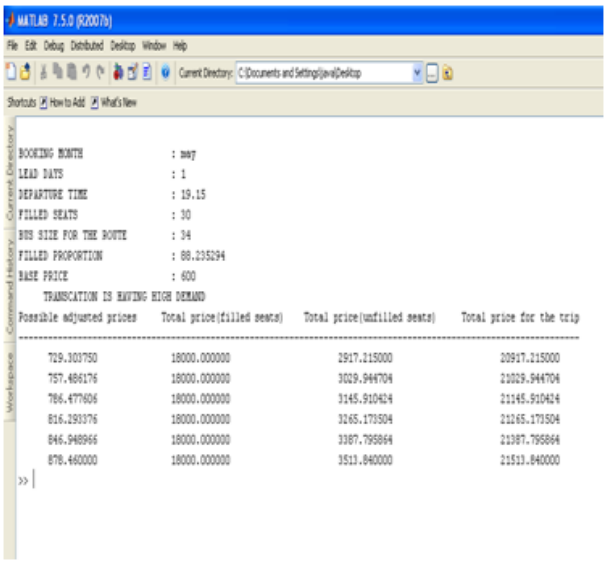


Fig 2. Calculating possible dynamic prices for high demand (for 2 day booked in advance)

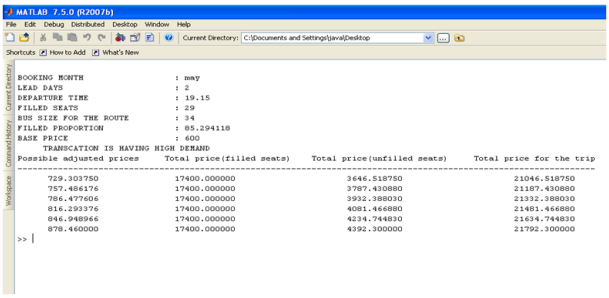


Fig 3. Calculating possible dynamic prices for moderate demand (for 1 day booked in advance)

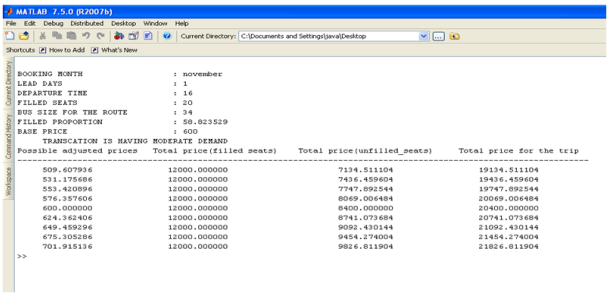


Fig 4. Calculating possible dynamic prices for moderate demand

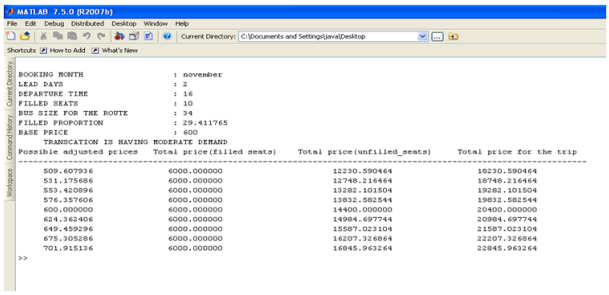
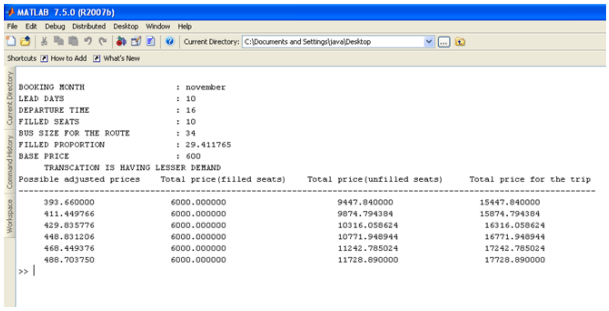


Fig 5. Calculating possible dynamic prices for lesser demand (for 10 days booked in advance)



5. CONCLUSION

As bus ticket pricing in the industry today is done manually by bus operators taking into consideration multiple factors and varies based on demand. We aimed at devising a dynamic pricing mechanism which will allow operators define their prices dynamically taking into consideration the market factors.

With the drawbacks mentioned above, price multipliers approach for dynamic pricing is developed and values are determined from the previous analysis. Final price is calculated with the determined price multiplier values. This computation is implemented through MATLAB and successfully tested for the route "Chennai-Mudalur 2012".

We firmly believe that a dynamic pricing approach will benefit both the customers and operators and hence will be immense value add to both.

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SLIGHTLY *g-CONTINUOUS FUNCTIONS

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ABSTRACT

In this paper we introduce slightly *gα-contoinuos function and investigated the properties of slightly *gα-continous functions. By utilizing *gα-open sets, we derived the theorem deals with covering properties and axioms.

Keywords: *gα-closed sets, *gα-open sets, slightly *gα-continuous, *gα-regular, *gα-normal , AMS Subject classification 54C10, 54C08, 54C05.

1. INTRODUCTION AND PRELIMINARIES

Continuous functions play an important role in the field of Mathematics. Large number of continuous functions have been introduced and their properties were investigated over the last two decades. Some of them are strongly α-irresoluteness (Fao, 1987) α-irresoluteness (Mashhour, *et al.,* 1983), α-continuity (Mashhour, 1983; Njastad, 1965), pre-continuity (Blumberg, 1992; Mashhour, 1982),semi-continuity (Levine, 1963), γ-continuity (El-Atik, 1997), slightly continuity (Jain, 1980; Singal and Jain, 1997)] and slightly γ-continuity (Eradal Ekici and Miguel Caldas, 2004).

The aim of this paper is to introduce slightly *gα-continuous functions and investigate the properties of slightly *gα-continuous functions. By utilizing *gα-open sets, we derive the theorems which deals with covering properties and separation axioms.

Throughout the present paper, X and Y are always topological spaces. Let A be a subspace of X. We denote the interior and closure of a set A by int(A) and cl(A), respectively.

1.1. Definition

A subset A of a space X is said to be α-open if A⊂int(cl(int(A))) (Mashhour, 1983). The complement of α-open set is closed.

1.2. Definition

The intersection of all α–closed sets of X containing A is called the α–closure of A and is denoted by αcl(A).

1.3. Definition

A subset A of a space X is called

1.gα-closed (Maki *et al.,* 1993) if αcl(A)⊆U whenever A⊆U and U is α-open and the complement of gα-closed set is gα-open.

2.*gα-closed (Devi and Vigneshwaran, 2007) if cl(A)⊆U whenever A⊆U and U is gα-open and the complement of *gα-closed set is *gα-open.

1.4. Definition

A function f: (X,τ)→(Y,σ) is called of (4) *gα-continuous of[2] if $f^{-1}(V)$ is *gα-closed in (x,τ) for every closed set V of (Y,σ).

The family of all * gα-open (resp. *gα-clopen and clopen) sets of X is denoted *gαO(X) (resp. *gαCO(X) and CO(X).

2. SLIGHTLY *GA-CONTINUOUS FUNCTIONS

2.1. Definition

A function f: (X,τ)→(Y,σ) is called

1.Slighly *gα-continuous at a point xϵX if for each clopen subset V in Y containing f(x),there exists a *gα-open subset U in X containing x such that f(U)⊂V.

2.Slightly *gα-continuous if it has this property at each point of X.

2.2.Theorem

Let (X,τ) and (Y,σ) be topological spaces . The following statements are equivalent for a function f: (X,τ)→(Y,σ):

1. f is slightly *gα-continuous.

2. for every clopen set V⊂Y, $f^{-1}(V)$ is *gα-open.

3. for every clopen set V⊂ *Y*, $f^{-1}(V)$ is *gα-closed.

4. for every clopen set V⊂ *Y*, $f^{-1}(V)$ is *gα-clopen.

Proof (1)⇒(2): Let V be a clopen subset of Y and let. Since f(x) ϵV by(1), there exists a *gα-open set U_x in X containing x such that $U_x \subset f^{-1}(V)$. We obtain $f^{-1}(V)=U\, x\epsilon f^{-1}(V)\, U_x$. Thus $f^{-1}(V)$ is *gα-open.

(2)⇒(3): Let V be a clopen subset of Y. Then Y\V is clopen. By (2) $f^{-1}(Y\backslash V)=X\backslash f^{-1}(V)$ is *gα-open $f^{-1}(V)$ is *gα-closed.

(3)⇒(4): Obivious.

(4)⇒(1): Let V be a clopen subset in Y containing f(x). By (4), $f^{-1}(V)$ is *gα-clopen. Take U= $f^{-1}(V)$. Then f(U) ⊂ V. Hence f is slightly *gα-continuous.

2.3. Theorem

If f: (X,τ)→(Y,σ) is slightly *gα-continuous and A∈ τ, then f\A : A→Y is slightly *gα-continuous.

Proof. Let V be a clopen subset of Y. We have $(f\backslash A)^{-1}(V) = f^{-1}(V) \cap A$. Since $f^{-1}(V)$ is *gα-open and A is open , then $(f\backslash A)^{-1}(V)$ is *gα-open in the relative topology of A. Thus f\A is slightly *gα-continuous.

2.4. Theorem

Let f: X→Y be a function and let g: X→XxY be the graph function of f, defined by g(x)=(x,f(x)) for every xϵX. Then g is slightly *gα-continuous if and only if f is slightly *gα-continuous.

Proof. Let VϵCO(Y), then XxV ϵCO(XxY). Since g is slightly *gα-continuous then $f^{-1}(V)= g^{-1}(XxV)$ ϵ*gαO(X). Thus f is slightly *gα-continuous.

Conversely, let xϵX and let W be a closed of XxY containing g(x). Then W∩({x}xY) is clopen in {x}xY containing g(x). Also {x}xY is homeomorphic to Y. Hence {yϵY\(x,y) ϵW} is clopen subset of Y. Since f is *gα-continuous, ∪{$f^{-1}(y)$|(x,y) ϵW} is *gα -open subset of X. Then xϵ ∪{$f^{-1}(y)$|(x,y) ϵW}⊂$g^{-1}(W)$. Hence $g^{-1}(W)$ is *gα-open. Then g is slightly *gα-continuous.

2.5. Definition

A function f: X→Y is called

1.*gα-irresolute if for every *gα-open subset A of Y, $f^{-1}(A)$ is *gα-open in Y.

2.*gα-open if for every *gα-open subset A of X, f(A) is *gα-open in Y.

2.6. Theorem

Let f: X→Y and g: Y→Z be functions. Then, the following properties hold:

1. If f is *gα-irresolute and g is slightly *gα-continuous, then g∘f: X→Z is slightly *gα-continuous.

2. If f is *gα-irresolute and g is slightly *gα-continuous, then g∘f: X→Z is slightly *gα-continuous.

3. If f is *gα-irresolute and g is slightly g-continuous, then g∘f: X→Z is slightly *gα-continuous.

Proof.

(1): Let V be any clopen set in Z. Since g is slightly *gα-continuous, then $g^{-1}(V)$ is *gα-open in Y. Since f is *gα-irresolute then $f^{-1}(g^{-1}(V))$ is *gα-open in X. Therefore g∘f is slightly *gα-continuous.

(3): Let V be a clopen set in Z. Since g is continuous, then $g^{-1}(V)$ is open in Y. Implies $g^{-1}(V)$ is *gα-open in Y. Since f is *gα-irresolute then $f^{-1}(g^{-1}(V))$ is *gα-open in X. Therefore g∘f is slightly *gα-continuous.

2.7. Theorem

Let f: X→Y and g: Y→Z be functions. If f is *gα-open and surjective and g∘f: X→Z is slightly *gα-continuous.

Proof. Let V be any clopen set in Z. Since g∘f is slightly *gα-continuous, $(g\circ f)^{-1}(V)= f^{-1}(g^{-1}(V))$ is *gα-open in X. Since f is*gα-open, then $f(f^{-1}(g^{-1}(V)))= g^{-1}(V)$ is is *gα-open in Y. Hence g is slightly *gα-continuous.

Combine the above two theorems, we get the following theorem.

2.8. Theorem.

Let f: X→Y be surjective, *gα-irresolute and *gα-open and g: Y→Z be a function. Then g∘f: X→Z is slightly *gα-continuous if and only if g is slightly *gα-continuous.

2.9. Definition

1. A filter base Λ is said to be *gα-convergent to a point x in X if for any U∈*gαO(X) containing x, there exists a B∈ Λ such that B⊂U.

2. A filter base Λ is said to be co-convergent to a point x in X if for any U∈CO(X) containing x, there exists a B∈ Λ such that B⊂U.

2.10. Theorem

If a function f: X→Y is slightly *gα-continuous then for each point x∈ X and each filter base Λ in X *gα-converging to x, the filter base f(Λ) is co-convergent to f(x).

Proof. Let x∈ X and Λ be any filter base in Λ in X *gα-converging to x. Since f is slightly *gα-continuous, then for any V∈CO(Y) containing f(x), there exixts a U ϵ*gαO(X) containing x such that f(U)⊂V. Since Λ is *gα-converging to x, there exists a B∈ Λ such that B⊂U. This means that f(B)⊂Vand therefore that filter base f(Λ) is co-convergent to f(x).

2.11. Definition (Devi and Vigneshwaran, 2007)

A space X is called *gα-connected provided that X is not the union of two disjoint non-empty *gα-open sets.

2.12. Theore.

If f: X→Y is slightly *gα-continuous surjective function and X is *gα-connected space, then Y is connected space.

Proof. Suppose that Y is not connected space. Then there exists non-empty disjoint open sets U and V such that Y=U∪V. Therefore, U and V are clopen sets in Y. Since f is slightly *gα-continuous, then $f^{-1}(U)$ and $f^{-1}(V)$ are *gα-closed and *gα-open in X. Moreover, $f^{-1}(U)$ and $f^{-1}(V)$ are non-empty disjoint and $X= f^{-1}(U) \cup f^{-1}(V)$. This shows that X is not *gα-connected. This is a contradiction. Hence Y is connected.

3. COVERING PROPERTIES

3.1.Definiition

1. A space X is said to be mildly compact (Stannum, 1974) if every clopen cover of X has a finite subcover.

2. A space X is said to be *gα-compact (Devi and Vigneshwaran, 2007) if every *gα-open cover has a finite subcover.

3. A subset A of a space X is said to be mildly compact relative to X if every cover of A by clopen sets of X has a finite subcover.

4. A subset A of a space X is said to be *gα-compact relative to X if every *gα-open sets of X has a finite subcover.

5. A subset A of a space X is said to be mildly compact if the subspace A is mildly compact.

6. A subset A of a space X is said to be *gα-compact if the subspace A is *gα-compact.

3.2. Theorem

If a function f: X→Y is slightly *gα-continuous and k is *gα-compact relative to X, then f(K) is mildly compact in Y.

Proof. Let $\{H_\alpha: \alpha\in I\}$ be any cover of f(K) by clopen sets of the subspace f(K). For each $\alpha\in I$, there exists a clopen sets K_α of Y such that $H_\alpha = K_\alpha \cap f(K)$. For each $x\in K$, there exists $\alpha_x\in I$,such that $f(x) \in K_{\alpha x}$. Since the family $\{U_x: x\in K\}$ is a cover of K by *gα-open sets of K, there exists a finite subset K_0 of K such that $K \subset\{U_x: x\in K_0\}$. Therefore, we obtain $f(K) \subset\cup\{f\ U(U_x): x\in K_0\}$ which is a subset of $\cup\{ K_{\alpha x} : x\in K_0\}$. Thus $f(K)= \cup\{ H_{\alpha x} : x\in K_0\}$and hence f(K) is mildly compact.

3.3. Corolary

If f: X→Y is slightly *gα-continuous surjective and X is *gα-compact then Y is mildly compact.

Proof. Similar to the above theorem.

3.4. Definition A space X is said to be

1. mildly countably compact (Stannum, 1974) if every clopen countably cover of X has a finite subcover.

2. mildly Lindelof (Stannum, 1974) if every cover of X by clopen sets has a countable subcover.

3. countably *gα-compact if every *gα-open countably cover of X has a finite subcover.

4. *gα-Lindelof if every *gα-open cover of X has a countable subcover.

5. *gα-closed compact if every *gα-closed cover of X has a finite subcover.

6.Countably *gα-closed compact if every countable cover of X by *gα-closed sets has a finite subcover.

7. *gα-closed Lindelof if every cover of X by *gα-closed sets has a countable subcover.

3.5. Theorem

Let f: X→Y be a slightly *gα-continuous surjection. Then the following statements hold:

1.if X is *gα-Lindelof, then Y is mildly Lindelof.

2.if X is countably *gα-compact, then Y is mildly countably compact.

Proof

(1): Let $\{V_\alpha: \alpha\in I\}$ be any clopn cover of Y. Since f is slightly *gα-continuous, then$\{f^{-1}(V_\alpha): \alpha\in I\}$ is a *gα-open cover of X. Since X is *gα-Lindelof, there exists a countable subset I_0 of I such that $X=\cup\{f^{-1}(V_\alpha): \alpha\in I_0\}$. Thus we have $Y=\cup\{V_\alpha: \alpha\in I_0\}$ and Y is mildly Lindelof.

(2): Similar to (1).

3.6. Theorem

Let f: X→Y be a slightly *gα-continuous surjection. Then the following statements hold:

1.if X is *gα-closed compact, then Y is mildly compact

2.if X is *gα-closed Lindelof, then Y is mildly compact

Proof. Similar of the above theorem.

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INVESTIGATION ON VARIOUS PARAMETERS OF SOAPS AND DETERGENTS

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ABSTRACT

Soap is a substance that dissolves in water to remove dirt from surfaces such as skin, textiles and other solids. Soaps are mainly used as surfactants for washing, bathing and cleaning. They are also used in textiles spinning and are important components of lubricants. The present study was carried out to assess the various properties of different soaps, in terms of pH, basicity, emulsification, content of fat, washing property, moisture content, foaming capacity and hard water reaction. A cursory look at the obtained results reveals similarities in parameters in the selected soaps It can been concluded that the values determined are within the limits set by standards.

Keywords : Soaps, Detergents, pH, TFM, hard water reactions, foam capacity.

1. INTRODUCTION

The name of soap, after an ancient Roman legend, comes apparently from Mount Sapo, where animals were slaughtered. The rain had mixed fat, tallow and ashes on the Tiber. The women found that this mixture enhanced the work, and started to use the slity soil, moistened with a mixture of fat. Soap, in fact, is the oldest active substances, and has been used about 4500 years. For centuries, soap was the only one cleaning substance available. Historically, it has been claimed that the esteem of a country's civilization is based on consumption of soap. In the 18th century, because of the shortage of some raw materials, soap was a highly priced luxury, and only wealthy people could afford it. It became handy to other people only after the manufacture of sodium carbonate was developed (Schulze, 1968). At the end of the 19th century, the first soap powder for laundry was made using sodium silicate as a builder. Whereas the use of sodium or potassium carbonate leads to a hard or soft soap, respectively. The chemical nature of the lipophilic part of the soap plays by far the largest role in determining the performance of the finished soap (Viorica Popescu *et al.,* 2011).

The first written record of soap can be seen in the writings of the Roman Pliny the Elder. He described the Phoenicians' synthesis of soap by using goat tallow and ashes. By the second centaury A. D, sodium carbonate was heated with lime (from limestone) to produce sodium hydroxide (lye). The sodium hydroxide was heated with animal fats or vegetable oils to produce soap (Bahl, 1991). Other societies made soap in much the same manner.

Soap (sodium salt of fatty acid) the oldest known surfactant has been used since the Egyptian era (Saad Moulay, 2011). It is now used mostly as toiletry soap bars for body cleansers and sometimes for fabric detergents and today for cleaning utensils. In detergents, soap is sometimes formulated as foam control agent. Direct saponification from fats was used initially to make soap but, today, soap is obtained by neutralizing fatty acids with NaOH. As the salt of a weak acid (fatty acid) and strong base (NaOH), soap is alkaline (pH 10) in aqueous solution, the alkalinity favours detergency but can cause skin irritation and hair damage. In addition, soap is not tolerant of hard water and forms scum with calcium and magnesium ions (Roila Awang *et al.,* 2001). In the present study the various properties of soap like pH, basicity, emulsification, content of fat, washing property, moisture content, foaming capacity and hard water reaction were evaluated.

2. MATERIALS AND METHODS

2.1. Preparation of Samples

The soaps analyzed were purchased from the departmental store. The bathing soap - Nature Power Herbal soap and Fair Beat, dish washing soap – Vim bar and Exo bar, fabric cleansing soap – Power detergent and Sundari detergent (Figure 1), were made into small bits using a steel scrapper and stored in different air tight container.

Figure. 1. Soap samples scrapped into bits

2.2. Determination of pH

10g of the powdered soap was weighed and dissolved in distilled water in a 100ml volumetric flask. This was made up to prepare 10% soap solution (Jahagirdar 1994, Dalen, 2009). The pH of the soap solution was determined using Model ELICO pH meter.

2.3. Determination of basicity

0.1g of soap samples was dissolved in 5ml of ethanol. A few drops of the phenolphthalein were added (Blackburn *et al.,* 1996). The appearance of the solution was noted.

2.4. Determination of Emulsification

100ml of coconut oil was taken in the test tube and 10ml of distilled water was added and shaken well. 0.1 gm of soap was added the above mixture. The appearance of the solution was noted.

2.5. Determination of Total Fatty Matter (TFM)

The soap samples were made into small slices. 5g of thoroughly mixed soap sample was weighed, using an electronic balance, the weight was noted. The weighed soap samples were taken into a separating funnel and 30 ml of hot water was added from a beaker and mixed well. Excess of 1:1 HCl was added to decompose the soap till acids floated as a clear layer on the top. 50ml of ether was added in the cold condition, mixed well and separated (Kundu *et al.,* 1977).

The aqueous layer was taken in another separating funnel and extracted with ether. The combined ether extracts were washed thrice with water to remove mineral acids and the contents were collected in a previously weighed 100ml conical flask. The ether was allow to evaporated (with the contents) in a hot air oven to constant weight.

From the difference in weight, the % of fatty matter in the analyzed soap samples was calculated using the relation (Viorica Popescu *et al.,* 2011).

Fatty matter (%) = (B-A)/C × 100

Where,

A - Weight of the empty conical flask

B - Weight of the conical flask + Soap after drying

C - Weight of initial sample of soap.

2.5.1. Determination of Washing Property

Each of the soap samples were used to wash the hands. The observations were noted.

2.5.2. Determination of Moisture Content

5g of the scrapped soap samples were weighed in an electronic balance into a pre weighed petri dish and placed in a hot oven at 105˚C. It was then placed in the crucible in the desiccator and its content was weighed after cooling. The moisture content was found from the weight difference (Mak-Mensa, 2011). The moisture content of the samples was calculated using the following equation.

% W= A – B / B × 100

Where, % W = Percentage of moisture in the sample,

A = Weight of wet sample (grams) and

B = Weight of dry sample (grams)

2.5.3. Determination of Foaming Capacity

6 conical flasks (100ml) were taken and numbered 1 to 6. In each of these flasks equal amounts (5g) of the samples of soap shavings were taken and 50ml of distilled water was added. Each conical flask was heated for few minutes to dissolve all the soap. In a test tube stand, six big clean and dry test tubes were taken and numbered them 1 to 6. One ml of the soap solution was then poured in to the test tubes of corresponding number. 10ml of distilled water was then added to each test tube. Test tube no. 1 was then shaken vigorously (5 times). The foam was found to be formed in the empty space above the container. Stop watch was started immediately and the time taken for the disappearance of foam was noted

Similarly the other test tubes were shaken vigorously for equal number of times (i.e., 5 times) with approximately with the same force and the time taken for the disappearance of foam in each case was recorded.

The lesser the time taken for the disappearance of foam, the lower is the foaming capacity. The soap for which the time taken for disappearance of foam is highest has maximum foaming capacity and is the best quality soap among the soaps tested.

2.5.4. Determination of Hard Water Reaction

1g of the powdered soap sample was taken and warmed with 50ml of water in a 100ml beaker. When the clear solution was obtained, it was poured in to each of three test tubes. One of the three test tube with 10 drops of 5% $CaCl_2$ solution, one with 10 drops of 5% of $MgCl_2$ solution and one with 10 drops of 5% $FeSO_4$ solution (Blackburn, 1996). These solutions stand until the other tests have finished. The observations were noted.

3. RESULTS AND DISCUSSIONS

Most soaps and cleaners usually remove dirt adequately, but their use is not devoid of adverse side-effects (Beetseh, 2013). These adverse effects include damage to the barrier function of the skin, increased susceptibility to environmental irritants and antigens, skin irritation with erythema and edema, and reduction of the cosmetic qualities of the skin, such as moisture and smoothness. These changes are usually subtle, occurring slowly over time, and are most important in elderly and atopic patients. Often, the association of these problems with the use of a particular type of soap is overlooked. Skin dryness can be exacerbated by dry climate and the influence of hard water, which increases the irritant effect of soaps or detergents (Lourdes Baranda *et al.,* 2002). Although it is important that the general population are aware of the potential of products used for body cleansing, fabric cleansing and dish washing, this information is not usually available therefore, it was decided to analyze the pH, foaming capacity, moisture content, basicity, hard water reaction, emulsification, content of fat and washing property.

3.1. pH of Soaps

Soap is a base and exact pH would be different depending on the soap. The pH level of soap can vary with the brand and with the function of the soap. However soap makers measure the acidity or alkalinity of soap by its pH level on a scale of 0 to 14. The average pH levels in bath soaps range from 3.61 to 12.38. For a general soap pH is from 5 to 8 and for hand soaps the pH is anywhere from 10 to 12. A 2002 International Journal of Dermatology study found pH levels in bath soap ranging 3.61 to 12.38. High pH soaps caused the most irritation. In liquid dish washing liquids, a pH in the range of about 7-8 (Dalen, 2009; Beetseh, 2013) .

The pH of the soap samples were determined using ELICO pH meter and it was found to be 7.3, 7.2, 8.1, 7.6, 7.8 and 7.7 for Nature power, Fair beat, Vim, Exo, Power and Sundari soap respectively. As soaps are more frequently used by general population, soaps with high irritation index should not used by individuals. The test samples had pH ranging from 7.3 to 8.1 which were well within the recommended levels.

3.2. Basicity of soaps

Soaps undergo a hydrolysis reaction in water. As a result, soap solutions tend to be alkaline. Detergents solutions on the other hand tend to be more neutral. Phenolphthalein indicator when in contact or presence of acid it will turn colorless and with base, it will turn into a pinkish violet color (Blackburn, 1996). When the solution of soap is treated with 2-3 drops of phenolphthalein, it is found to show no change, indicating that the selected soaps are found to be neutral.

3.3. Emusification of soaps

Soap is an excellent cleanser because of its ability to act as an emulsify agent. An emulsifier is capable of dispersing one liquid into another immiscible liquid. This means that while oil (which attracts dirt) does not naturally mix with water, soap can suspend oil/dirt in such a way that it can be removed.

For a soap to perform its cleaning activity it should be able to form emulsion in presence of oil. As a result of their molecular structures, soaps and detergents are both capable of emulsifying or dispersing oils and similar water-insoluble substances. Results showed that all the selected soap samples were found to have good cleansing action as they formed emulsion when shaken with oil.

3.4. Total Fatty Matter (TFM) of soaps

Total Fatty Matter (TFM) is one of the most important characteristics describing the quality of soap and it is always specified in commercial transaction. It is defined as the total amount of fatty matter, mostly fatty acids, that can be separated from a sample after splitting with mineral, usually hydrochloric acid. The fatty acids most commonly present in soap are oleic, stearic and palmitic acids and pure, dry, sodium oelate has TFM 92.8%, while top quality soap noodles now increasingly used for making soap tables in small and medium size factories, are typically traded with a specification TFM 78% minimum, moisture 14% maximum. But besides moisture, finished commercial soap, especially laundry soap, also contains fillers used to lower its cost or confer special properties, plus emollients, preservatives.etc. and then the TFM can be as low as 50%. Fillers which are usually dry powders, also make the soap harder, harsher on the skin and with greater tendency to become 'mushy' in water and so low TFM is usually associated with hardness and lower quality. In older days in Europe and in some countries, soap with TFM 75% minimum was referred to as Grade I and 65% minimum as Grade2 and less 60% Grade 3.

However according to the norms laid down by Bureau of Indian Standards (BIS) soaps are graded into three categories. Soaps containing TFM of 76% and above are Grade 1. Soaps with TFM content of 70% and above but less than 76% are considered to be Grade 2 soaps and soaps having

TFM content of 60% and above but less than 70% are graded as Grade 3.

Grade 2 and 3 soaps contain higher amount of fillers. Sometimes these fillers may even contain asbestos which can have an adverse effect on skin upon continued usage. They have tendency of getting mushy in water and tend to get consumed faster. Lower grade soaps have poorer lathering.

Grade 1 soaps on the other hand have a higher TFM content and provide very high cleansing efficiency while being gentle on skin.

Soap which is basically a cleansing product can be thus categorized based on it TFM content. Simply put, higher the TFM of soap better is its cleansing ability. Table 7 shows the TFM values of the test samples. All selected brands comply with the minimum requirement of total fatty matter as per the standard. All the selected sample except sundari soap can be categorized as grade 1 soap (Beetseh, 2013).

3.5. Washing property of soaps

In everyday life soap are used to wash dishes, clean clothes or keep our body presentable. Soap therefore has numerous applications in our daily life. One of its great values is keeping the hands out of significant risk (Warra, 2013). However the main purpose of soap is lost when these substances induce skin irritation and injury. Most soaps and detergents are alkaline and induce an increase in cutaneous pH. In addition, repeated washing with soap may reduce the normal skin flora, leading to an increased colonization of the skin with coagulase-negative staphylococci; this effect has been linked to the skin pH caused by soaps (Mirela Moldovan, 2010; Lourdes Baranda *et al.,* 2002).

3.6. Moisture content of soaps

Soaps often require a precise quantity of moisture in order to work at their most efficient levels; too little moisture leaves the soap too dry to use, while excessive moisture will make solid soaps more difficult to unmold and dry out. As common sense would dictate, one would expect a high level of moisture in liquid soap products. Moisture content affects soap making. The process of making soap (called "saponification") is highly dependent on the moisture level in the mixture (Simmons and Appleton, 2007). A major chemical ingredient in soap-making is alkali, such as lye, which reacts with fatty acid esters to produce neutral salts of fatty acids, the principal ingredient in soaps. The lower the water content of the reaction mixture, the higher the pH and the stronger the alkali. If the moisture level in the saponification reaction mixture is too high, the alkalinity will be reduced and the reaction will proceed too slowly. However, a high concentration of alkali chemicals is corrosive and damaging to people and equipment and must be handled with care. If the soap recipe does not have enough water the solid soap produced may form cracks. The moisture content of solid soap is adjusted by evaporation after the chemical reaction is complete.

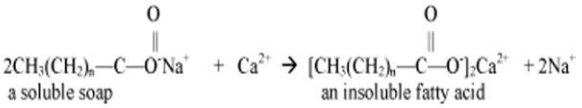
There is no specific measurement of moisture content as per the national standard. It should not be too high or too low (A report of Consumer voice, 2014). Exo and Nature power contained the lowest moisture followed by Fair beat and sundari soap. Vim and power soap contained the maximum moisture.

3.7. Foaming Capacity of Soaps

Lather is the foam or the forth created by soap when stirred in water or while bathing or washing hands. It is an important parameter for acceptability of soaps. All the brands passed in the lather test. Fair beat alone was found to produce thick rubbery foam. The foam stability was determined by noting the time taken for disappearance of 2mm of froth. From Table. 2 Fair Beat was found to have high foaming capacity of 3 minutes and 40 seconds. Power detergent soap showed the lowest foaming capacity of 1 minute.

3.8. Hard water reaction of soaps

The sodium and potassium salts of most carboxylic acids are water soluble. However, the calcium, magnesium, and iron salts are not. Thus when soaps are placed in hard water that contains such ions, an insoluble, curdy solid is formed. Often it has been seen that these results in the form of a bathtub ring or soap scum floating in bath or wash water. This process removes soap ions from solution, and decreases the cleaning effectiveness of soaps.



The calcium, magnesium, and iron forms of most detergents are more soluble in water than the corresponding soaps compounds (Blackburn, 1996). Consequently, detergents function almost as well in hard water as they do in soft water.

The selected soaps formed scum immediately / slowly in plenty / in scanty. All the soap samples formed scum when treated with $CaCl_2$ and $FeSO_4$. While only Nature Power and Fair beat

formed scum with $MgCl_2$. The results (Table. 3) show that Vim, Exo, Power and Sundari saop can be used even in hard water for cleaning purpose.

In the present study the effect of the soaps on the hands were determined by washing the hands with the selected soaps. All the soap samples were found to be soft on hand. It can be concluded these soaps are safe for use by public.

The results were sufficiently promising to warrant further investigation.

4. CONCLUSION

All the samples were observed to be good cleansers.

- pH of soap samples were found to be in the neutral range 7.2 – 8.1.
- All samples were found to have neutral basicity.
- All the soap samples formed emulsion when shaken with oil.
- The TFM values ranged from 63 to 80 which were well within the standard values
- All the soap samples were found to be soft on hand while washing.
- Moisture content was in the range 6.50% to 22.90%.
- Fair beat soap showed high foaming capacity of 3 40' minutes. Power detergent soap showed the lowest foaming capacity with 1 minute as the time taken for disappearance of 2mm of soap froth.
- In hard water reaction all the soap samples formed scum when treated with $CaCl_2$ and $FeSO_4$ while only Nature power and Fair beat formed scum with $MgCl_2$.

The results further indicate that good soaps are not characterized by their fragrance or appearance, nor by the place in which they are sold, but by their properties the exhibit.

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Table 1. Different Parameters of soap samples

Soap sample	pH	Basicity	Emulsification	TFM	Washing property	Moisture content (%)
Nature Power	7.3	Neutral	Emulsion formed	77	Soft	9.60
Fair beat	7.2	Neutral	Emulsion formed	80	Soft	12.40
Vim	8.1	Neutral	Emulsion formed	78	Soft	22.90
Exo	7.6	Neutral	Emulsion formed	79	Soft	6.50
Power	7.8	Neutral	Emulsion formed	75	Soft	21.78
Sundari	7.7	Neutral	Emulsion formed	63	Soft	18.74

Table 2. Foaming capacity of soap samples

Name of the soap sample	Volume of soap solution (ml)	Volume of water added (ml)	Time taken for disappearance of 2mm of soap froth	
			Minutes	Seconds
Nature power	1	3	3	2
Fair beat	1	3	3	40
Vim	1	3	2	00
Exo	1	3	2	10
Power	1	3	1	00
Sundari	1	3	1	20

Table 3 Hard water reaction of Soap samples

S.No.	Name of the soap sample	Hard water reaction		
		$CaCl_2$	$MgCl_2$	$FeSO_4$
1	Nature Power	Scum formed in plenty	Scum formed very slowly and scanty	Scum formed immediately and plenty
2	Fair Beat	Scum formed slowly and plenty	Scum formed very slowly and scanty	Scum formed immediately and plenty
3	Vim	Scum formed and Scanty	No scum formed	Scum formed immediately and plenty
4	Exo	Scum formed and Scanty	No scum formed	Scum formed immediately and plenty
5	Power	Scum formed and Scanty	No scum formed	Scum formed slowly and scanty
6	Sundari	Scum formed slowly and plenty	No scum formed	Scum formed slowly and scanty

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PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL STUDIES OF SOME HYDRAZINIUM CARBOXYLATES

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ABSTRACT

Some new hydrazinium salt of aromatic carboxylic acids have been prepared by neutralization of acid with hydrazine hydrate in aqueous medium and characterized by Analytical, IR spectral and TG-DTA analysis. All compounds undergo two, three or four step decomposition yielding carbon residue as the final product. The in vitro antibacterial screening of 2,4-dichlorophenoxyacetic acid and its hydrazinium salt against *Escherichia coli* have been investigated. The antibacterial activity of the prepared hydrazinium salt shows more promising activity than the free acid.

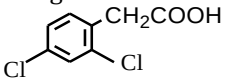
Keywords: Hydrazinium salt, Aromatic carboxylic acids, IR spectral.

1. INTRODUCTION

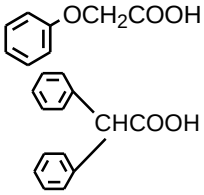
Hydrazine is the simplest diamine and forms salt with mineral and carboxylic acids (Vogel, 1962; Yasodhai and Govindarajan, 1999). The preparation of hydrazinium salts has become a subject of recent interest due to their wide use as additives in propellants, drugs to treat cancer and Hodgkin's diseases and explosives (Vogel, 1962). They have also been used as ligands for the preparation of metal hydrazinium/hydrazine complexes (Patil *et al.,* 1980, Govindarajan *et al.,* 1986 a and b; Chandra and Singh, 1983). Some of these salts are used as flame – retardants (Schmidt, 1984; Balague *et al.,* 2001) and proton conductors (Patil *et al.,* 1979). Only few of these salts show antibacterial activity (Govindarajan, *et al.,* 1980). Preparation and thermal behaviour of some salts from few aliphatic acid (Patil *et al.,* 1981) and aromatic carboxylic acids (Allan, *et al.,* 1998; Vairam and Govindarajan, 2004) have been reported. There is no literature citations about hydrazinium salt of aromatic substituted acetic acids, aromatic unsaturated acids and hetero acids except hydrazinium salt of pyrazine mono and di-carboxylic acids (Premkumar and Govindarajan, 2006). It is therefore, considered interesting to prepare hydrazinium salt of aromatic substituted acetic acids namely 2,4-dichlorophenylacetic acid, phenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, and Diphenylacetic acid, aromatic unsaturated acid namely cinnamic acid, hetero acids namely picolinic acid and nicotinic acid.

Generally all phenolic derivatives show antibacterial property. Particularly 2,4-dichlorophenoxyacetic acid altered envelope properties of the bacteria Escherichia coli, such as hydrophobic index Unsubstituted phenoxyacetic acid is also a phenolic derivative. But, it has no potent substituents to have antibacterial property. 2,4-dichlorophenoxyacetic acid contain two potent chloro substituents. It influences it to have antibacterial property like chloroxylenol (4-chloro-2,5-Xylenol) which acts as antiseptic as well as disinfectants. This prompted us to make antibacterial study of hydrazinium salt of 2,4-dichlorophenoxyacetic acid against *Escherichia coli*

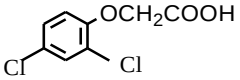
The structures of acids and their designations used are shown below for clarity



2,4-dichlorophenylacetic acid

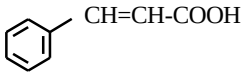


phenoxy acetic acid

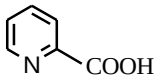


2,4-dichlorophenoxyacetic acid

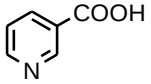
Diphenylacetic acid



Cinnamic acid



Picolinic acid



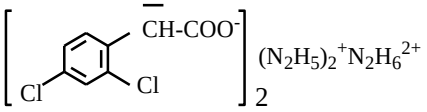
Nicotonic acid

2. EXPERIMENTAL

All the salts reported have been prepared by the neutralization of the respective carboxylic acids in aqueous medium with hydrazine hydrate (99 – 100 %) in appropriate molar ratios.

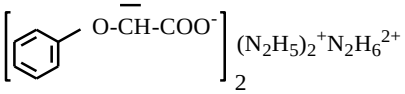
2.1. Preparation

2.2.1. Hydrazinium 2,4-dichlorophenyl acetate



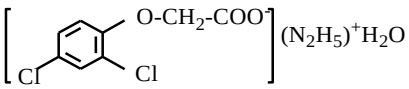
This is prepared by mixing hydrazine hydrate and 2,4-dichlorophenylacetic acid in 2:3 molar ratio in 50 mL of distilled water. The resulting turbid solution is heated over water bath to obtain clear solution and concentrated to nearly 20 mL. Then it is allowed to crystallize at room temperature. Light yellow coloured hydrazinium salt is crystallized out after 24 hours. The crystals are filtered off and washed by using benzene and dried in air.

2.2.2. Hydrazinium phenoxyacetate



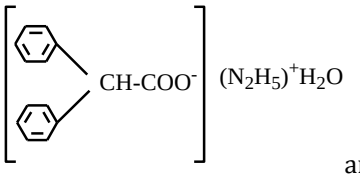
This is also prepared by the same procedure as above by mixing the acid with the base in the molar ratio of 2:3. Spongy white coloured salt is crystallized within 20 minutes. The product is washed by using benzene and air dried.

2.2.3. Hydrazinium 2,4-dichlorophenoxy acetate

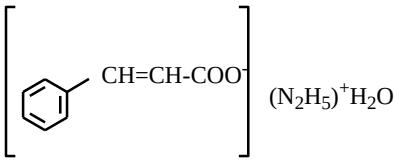


The hydrated salt is also prepared by the same procedure by mixing hydrazine hydrate and 2,4-dichlorophenoxyacetic acid with molar ratio 1:1. White coloured salt is crystallized out immediately. This is washed by using alcohol and air dried.

2.2.4. Hydrazinium diphenyl acetate hydrate and hydrazinium cinnamate hydrate

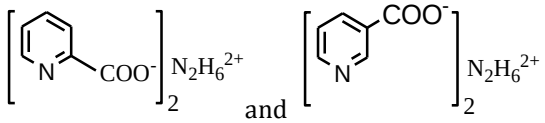


and



These are prepard by mixing hydrazine hydrate and Diphenyl acetic acid or cinnamic acid in 1:1 molar ratio in 50 mL of water. The contents of the beaker are heated on water bath. Then the undissolved acid is removed by filtration. The resulting clear solution is concentrated on water bath to 20 mL. Then it is allowed to crystallize at room temperature. While the white coloured monohydrated hydrazinium(+1) salt of cinnamic acid separated out after 24 hours, whereas light yellow coloured monohydrated hydrazinium (+1) salt of Diphenylacetic acid separated out after 48 hours, they are filtered off and washed by using alcohol and air dried.

2.2.5. Hydrazinium picolinate and Hydrazinium nicolinate



These are prepared by mixing hydrazine hydrate and picolinic acid or nicotinic acid with 1:1 molar ratio in 50 mL of distilled water. The resulting clear solution is concentrated on water bath to 20 mL. Then it is allowed to crystallize in a vacuum desiccator over calcium chloride. Light yellow coloured hydrazinium(+2) salt of picolinic acid separated out after 2 days, filtered and washed by using alcohol, whereas white coloured hydrazinium(+2) salt of nicotinic acid is also separated out after 2 days, filtered and washed by using ether.

2.2.6. Physico-Chemical techniques

The hydrazine content of these salts are determined volumetrically using a standard (0.025 m) KIO_3 solution under Andrew's condition [17]. IR spectra are recorded as KBr pellets with a Shimadzu spectrophotometer in the range 4000-400 cm^{-1}. Elemental analyses are performed on a Perkin-Elmer 240 B CHN analyzer. Simultaneous TG-DTA measurements are carried out using STA 1500 thermal analyzer. All thermal analyses are carried out in air at a heating rate of 10°C per minute. Platinum cups are used as sample holders and alumina as reference.

The microorganism used to test the biological potential of 2,4-dichlorophenoxyacetic acid and its hydrazinium(+1) salt is Escherichia coli, obtained from the stock cultures of the Biotechnology Laboratory of the Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore, India.

2.2. Antibacterial activity

The antibacterial activity of 2,4-dichlorophenoxyacetic acid and its hydrazinium salt are determined by the disc diffusion method

(Cruickshank, 1968). The bacteria are cultured in nutrient agar medium and used as inoculum for the study. Bacterial cells are swabbed on to nutrient agar medium (prepared from NaCl (5.0g), peptone (5.0g), beef extract powder (3.0g), yeast extract powder (3.0 g), agar (20.0 g) in 100 mL distilled water, pH = 7.5 ± 0.2) in Petri dishes. The test solutions are prepared in distilled water to a final concentration of 2% and 4% and then applied to filter paper discs (Whatmann/No. 4.5 mm dia). These discs were placed on the already seeded plates and incubated at 35±2°C for 24hr. the zone of inhibition around the discs are measured after 24hr. Co-trimoxazole is used as a standard positive control.

3. RESULTS AND DISCUSSION

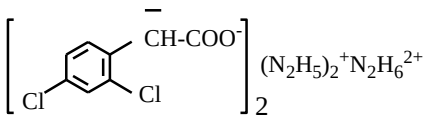
The analytical data of the salts (Table 1) are consistent with the proposed formulae for them.

3.1. Infrared spectra

The important IR bands of the salts are listed in Table 2. The IR spectra of the hydrated salts display absorption bands in the region of 3346 - 3330 cm^{-1} due to O-H stretching of water molecule. The bands in the region of 1390-1323 cm^{-1} and 1598-1521 cm^{-1} for these salts are corresponds to symmetric and asymmetric stretching frequencies of the carboxylate ions. The N-N stretching frequencies of $N_2H_5^+$ ion appear in the range of 963-951 cm^{-1} and the N-N stretching frequencies of $N_2H_6^{2+}$ ion shows bands in the region of 1047 - 1026 cm^{-1}.

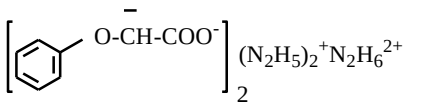
3.2. Thermal analysis

3.2.1. Hydrazinium 2,4-dichlorophenyl acetate



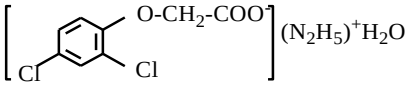
The thermogram of this salt indicates that the decomposition of the salt takes place in multi steps. In the first step, dehydrazination with melting takes place with endothermic peak at 99°C. In the second step the carboxylate intermediate decomposes to phenol with endothermic peak at 211°C and an exothermic peak at 269°C. In the next step showing exothermic peak at 305°C and 461°C due to the formation of formic acid. Finally it decomposes to carbon residue at 546°C.

3.2.2. Hydrazinium phenoxy acetate



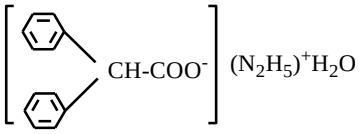
This undergoes decomposition in three steps. In the first step, compound undergoes melt with endothermic peak at 99°C. Then the compound decomposes to diphenyl glycol with exothermic peak at 149°C and 265°C. Finally at 327°C it decomposes to carbon residue.

3.2.3. Hydrazinium 2,4-dichlorophenoxy acetate hydrate



The thermogram of this salt indicates that the decomposition of the salt takes place in two steps. In the first step, both dehydration and dehydrazination occur simultaneously showing a sharp endotherm at 170°C. In the second step the acid intermediate decomposes exothermally at 285°C and 488°C to carbon residue.

3.2.4. Hydrazinium diphenyl acetate hydrate

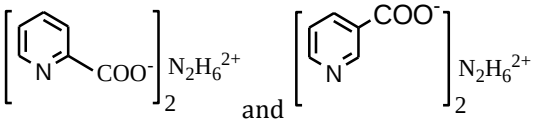


This undergoes decomposition in three steps. First step shows an endothermic peak at 106°C corresponding to the removal of moisture. In the second step, both dehydration and dehydrazination occur simultaneously showing two exothermic peaks at 186°C and 233°C to form formic acid. In the final step, formic acid completely decomposes to carbon residue.

3.2.5. Hydrazinium cinnamate hydrate

This compound also undergoes three steps decomposition. First step shows an endothermic peak at 92°C corresponding to liberation of water molecule. Second step shows on endothermic peak at 150°C due to the elimination of one molecule of hydrazine. In the third step complete decomposition to carbon residue takes place.

3.2.6. Hydrazinium picolinate and hydrazinium nicolinate



Both compounds undergo two step decomposition. First step is the removal of moisture with endothermic peaks at 121°C and 117°C respectively. In the second step, the compound completely decomposes to carbon residue.

TG – DTA curves of some compounds are given (Fig 1 - 4) as representative examples.

3.3. Antibacterial activity

The antibacterial activity of the 2,4-dichlorophenoxyacetic acid and its salt are determined by disc diffusion method. From the result (Table 4) it has been observed that hydrazinium salt of 2,4-dichlorophenoxyacetic acid shows more activity than the free acid.

4. CONCLUSION

2,4-dichlorophenylacetic acid and Phenoxyacetic acid form peculiar type of hydrazinium salts similar to double salts. These type of salts are not been reported so far in the literature. They contain both hydrazinium(+1) and hydrazinium(+2) ions. Their compositions are confirmed by analytical, IR spectral and Thermal studies. In these salts 'CH_2' group loses H^+ ion because of the presence of more electronegative groups on both sides and becomes carbanion and their charges are compensated by the extra hydrazinium(+2) ions. Phenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, Diphenylacetic acid and Cinnamic acid form salts containing only hydrazinium(+1) ions, whereas picolinic and nicotinic acid formed as hydrazinium(+2) salts.

All compounds undergo two, three or four step exothermic or endothermic decomposition through various intermediates. The double salts have more lattice enery than the other salts. Therefore they undergo melting before decomposition as observed in TG – DTA analysis, whereas the other simple salts decomposes before melting. All the salts decompose completely to give carbon residue as the final product.

The antibacterial activity of 2, 4-dichlorophenoxyacetic acid and its hydrazinium salt against *Escherichia coli*

has been studied. The antibacterial activity of the hydrazinium salt show more promising activity than the free acid.

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Table 1. Analytical data

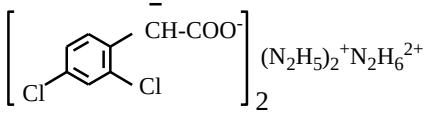
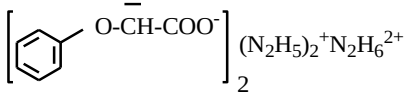
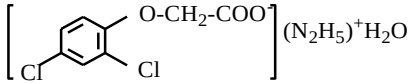
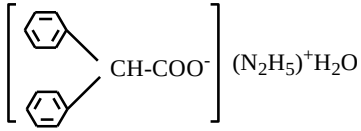
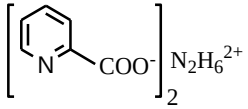
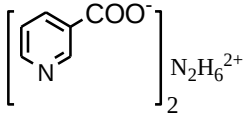
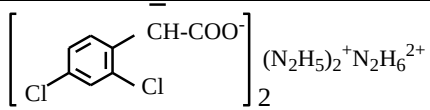
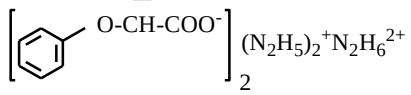
S.No	Compound	Colour	Found(calculated) %			
			Hydrazine	Carbon	Hydrogen	Nitrogen
1		Light yellow	19.20(19.47)	37.40(37.70)	4.50(4.91)	16.30(16.49)
2		White	24.19(24.70)	47.20(47.82)	7.11(7.20)	20.14(20.92)
3		White	11.21(12.14)	47.10(47.04)	4.10(4.41)	8.80(8.82)
4		Light yellow	12.00(12.55)	63.20(63.80)	6.20(6.83)	10.59(10.63)
5		White	17.40(17.19)	51.00(51.19)	7.04(7.02)	14.85(14.05)
6		Light yellow	12.71(12.10)	51.29(51.38)	2.89(2.85)	9.96(9.99)
7		White	12.83(12.10)	51.20(51.38)	2.80(2.85)	9.95(9.99)

Table 2. IR spectral data (cm^{-1})

S.No	Compound	υ_{OH} of water/acid	υ_{N-H}	υ_{asymm} (OCO)	υ_{sym} (OCO)	υ_{N-N}
2		-	3276 3257	1590	1380	958 1030
4		-	3443(b)	1589 1541	1375 1338	959 1026 1047

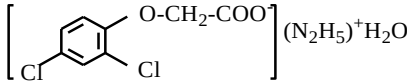
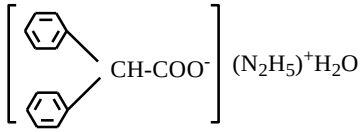
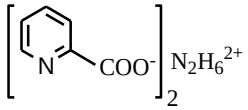
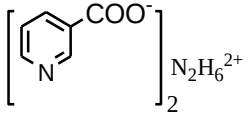
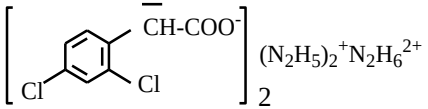
6		3330(s)	3172 3072	1577 1569	1390 1328	951
8		3346(s) 3331(s)	3276 3245	1596 1527	1375 1340	963
10		3332(b)	3251 3236	1558 1521	1386 1338	953
11		-	3276 3213	1583 1560	1385 1331	1043
12		-	3265 3246	1598 1544	1386 1323	1029

Table 3. TG - DTA data

S.No	Compound	DTA peak temp(°C)	Thermogravimetry(TG)			Decomposition product
			Temp range °C	Mass loss (%)		
				Observed	Calculated	
1		99(+) 211(+) 269(-) 305(-) 461(-) 546(-)	25-133 133-286 286-329 329-585	5.18 79.43 90.38 95.86	6.49 81.54 90.91 -	Melting and dehydrazination Decomposition to phenol Decomposition to formic acid Decomposition to carbon residue

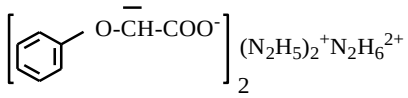
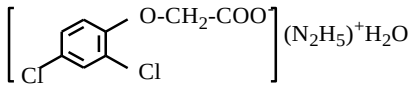
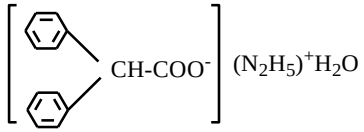
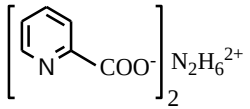
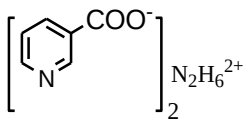
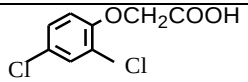
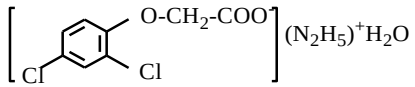
2		99(+) 149(-) 265(-) 327(-)	25-90 90-234 234-344	- 50.41 97.6	- 47.00 -	Melting Decomposition leads to diphenyl glycol Decomposition to carbon residue
3		170(+) 285(-) 488(-)	103-225 225-528	18.21 96	18.39 -	Dehydration and dehydrazination Complete decomposition to carbon residue
4		106(+) 186(-) 233(-) 484(-)	25-86 86-354 354-483	1 81.51 97	- 82.53 -	Removal of moisture Dehydration and dehydrazination leads to formic acid Complete decomposition to carbon residue
5		92(+) 150(+) 289(-) 458(-)	25-102 102-150 150-481	9.67 25.04 98.41	9.03 26.13 -	Dehydration dehydrazination Decomposition to carbon residue
6		121(+) 155(-) 191(+)	25-99 99-218	- 87.70	- -	Removal of moisture Decomposition to carbon residue
7		117(+) 183(-) 282(+)	25 – 127 127 - 304	3.69 90.20	- -	Removal of moisture Decomposition to carbon residue

Table 4. Antibacterial activity of 2,4-dichlorophenylacetic acid and its hydrazinium salt (The test solution is prepared in distilled water)

S.No	Compound	Diameter of inhibition zone (mm)	
		2 %	4%
1.		7	10
2.		19	26

Diameter of Zone of inhibition is a mean of triplicates

Fig 1 TG – DTA of 

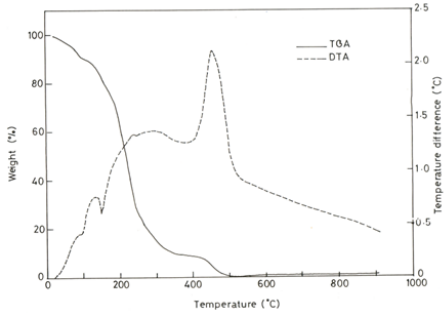
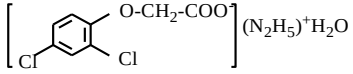


Fig 2 TG – DTA of 

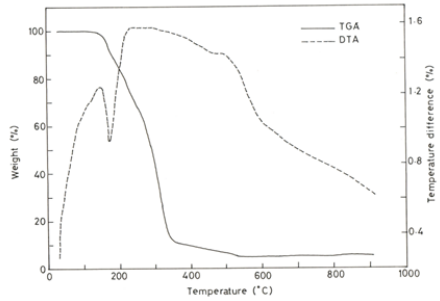
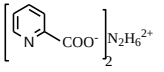


Fig 3 TG – DTA of 

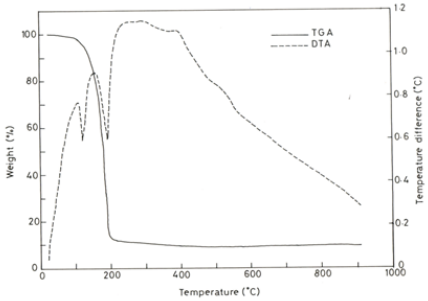
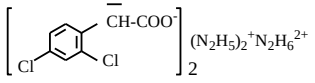
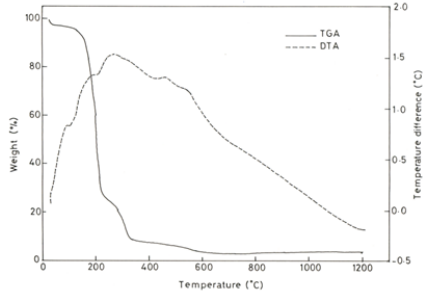


Fig 4 TG – DTA of 



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SYNTHESIS AND CONFORMATIONAL STUDIES ON CERTAIN *N*-NITROSO PIPERIDIN-4-ONES

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ABSTRACT

Heterocyclic compounds gain importance owing to their pharmacological, agro- hemical and in brief, biological activities. The piperidin-4-one units are present in a variety of alkaloids which are occurring naturally. They find wide applications as drugs. Further, the stereochemical studies of piperidinone chemistry are thought provoking and quiet interesting.

Keywords: *n*-nitroso piperidin-4-ones, Heterocyclic compounds, pharmacology.

1. INTRODUCTION

Piperidones are an important group of heterocyclic compounds in the field of medicinal chemistry due to their broad spectrum of biological activities. One such class of compounds containing 4-piperidones and their derivatives, whose synthesis and stereodynamics are well investigated (Prostokov and Gaivoronskaya, 1978). Many natural products and drugs contain the piperidine ring system as a structural element. Nitrogen heterocycles, in particular 4-piperidones display important biological properties such as antiviral, antitumor, analgesics and antihypertensive activities (Miyoshi *et al.,* 1995; Riley *et al.,* 1973). The importance of 4-piperidones as intermediates in the synthesis of a variety of compounds of physiological activity has been reviewed by Prostokov and Gaivoronskaya (Shintani *et al.,* 2004). The extensive studies undertaken in the past on 4-piperidones have their relation to the synthesis of drugs (Boach *et al.,* 1948)The utility of 2-aryl, 2-heteroarylpiperidin-4-ones in the construction of polycyclic systems such as benzo[*a*]quinolin-4-ones, indole alkaloids, have been disclosed by Rubiralta *et al.,* 1989 recently in a series of papers.

They have also described the importance of the introduction of bulky substituent in the nitrogen side of 4-piperidones, thereby making the ring system to adopt favorable conformation for the intramolecular ring closure leading to the construction of benzomorphon related compounds. Piperidone derivatives have also been noted to act as potential inhibitors of human placental aromatase *in vitro*. 3,5-bis(arylidine)piperidin-4-ones behave as cytotoxic and anticancer agents. 2,2,6,6-tetramethylpiperidin-4-one hydrochloride has been used as a spin trap in several EPR studies and it's hydrazones are used as antioxidants. 2-Aryl piperidin-4-ones are used as key intermediates for the synthesis of techykinin antagonists and indolizidine alkaloids (Boach *et al.,* 1948).

2. EXPERIMENTAL SECTION

Melting points of all the compounds were determined on an electrically heated block (RAAGA make) with a calibrated thermometer and are uncorrected. The IR spectra were recorded on a FTIR instrument (Perkin-Elmer). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer and 2D NMR spectra were recorded on a AV 500 MHz instrument in $CDCl_3$ solution with TMS as an internal standard.

2.1. Synthesis of r-2,c-6-Bis(2-chlorophenyl)-c-3,t-3-dimethylpiperidin-4-one (1)

To a solution of ammonium acetate (0.05 mole) in dry ethanol, 2-Chlorobenzaldehyde (0.1 mole) and isopropylmethyl ketone (0.05 mole) was added. The above contents were taken in a round bottom flask and fitted with a double walled condensor. It was heated for 30 minutes. Then it was kept at room temperature overnight. The formed crystals of *r*-2,*c*-6-bis(2-chlorophenyl)-*c*-3,*t*-3-dimethylpiperidin-4-one was filtered and washed well with the dry alcohol. Yield : 11.5 g (75%) m.p: 148^{o} C-150^{o} C & MS (m/z) : 347.31(M^{+}), 276.14, 252.06 (100%), 149.22, 129.28, 115.18, 69.24

2.2. Synthesis of r-2,c-6-Bis(2-chlorophenyl)-t-3,t-5-dimethylpiperidin-4-one (2)

To a solution of ammonium acetate (0.05 mole) in dry ethanol, 2-chlorobenzaldehyde (0.1 mole) and diethyl ketone (0.05mole) was added. The above contents were taken in a round bottom flask and fitted with a double walled condensor. It was heated for 30 minutes. Then it was kept at room temperature overnight. The crystals of *r*-2,*c*-6-bis(2-chlorophenyl)-*t*-3,*t*-5-dimethylpiperidin-4-one was separated was washed well with the dry alcohol.

Yield : 12.51 g (82%) m.p. : 117° C-120° C MS (m/z): 347.14(M^+), 312.04, 252.08 (100%), 152.21, 125.16, 117.25, 73.19

2.3. Synthesis of r-2,c-6-Bis(2-chlorophenyl)-t-3-methylpiperidin-4-one (3)

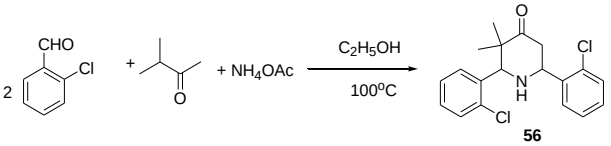
To a solution of ammonium acetate (0.05 mole) in dry ethanol 2-chlorobenzaldehyde (0.1 mole) and ethylmethyl ketone (0.05 mole) was added. The above contents were taken in a round bottom flask and fitted with a double walled condensor. It was heated for 30 minutes. Then it was kept at room temperature overnight. The crystals of r-2,c-6-bis(2-chlorophenyl)-t-3-methylpiperidin-4-one was separated out was filtered and washed well with the dry alcohol. Yield : 7.6 g (53%) m.p.: 124° C-126° C

3. RESULTS AND DISCUSSION

In the present work, *r*-2,*c*-6-bis(2-chlorophenyl)piperidin-4-ones **1 & 2** and their corresponding *N*-nitroso compounds **4 & 5** respectively, have been synthesized and their stereochemistry studied using IR spectra, ^{1}H & ^{13}C and 2D (^{1}H, ^{1}H–COSY & ^{1}H, ^{13}C-HETCOR) NMR Spectra. The NMR spectral data reveal that all the parent piperidin-4-ones **1 & 2** prefer chair conformation while the *N*-nitroso compounds **4 & 5** prefer to exist in a twist-boat conformation with coplanar orientation of *N*-N=O moiety.

3.1. r-2, c-6-bis(2-chlorophenyl)c-3, t-3-dimethyl-piperidin-4-one (1).

The piperidin-4-one **1** was synthesized by the reaction of isopropyl methyl ketone, 2-chlorobenzaldehyde and ammonium acetate in ethanol medium at 100 °C (**Scheme 11**).



Scheme 1

The structure of the compound **1** was confirmed by IR spectra, ^{1}H, ^{13}C NMR, 2D NMR and mass spectral data .

The IR spectrum of piperidin-4-one **1** , showed the presence of >NH (stretching band observed at 3306 cm^{-1}) and >C = O (stretching band observed at 1703 cm^{-1}) groups, which confirmed the formation of the compound **1**.

The ^{1}H NMR signals of the compound **1** were assigned by comparison with those of the corresponding 2,6-bis (2-clorophenyl) -3-methyl piperidin -4- one (**3**). The signal integration values were also used for the assignment.

The ^{1}H NMR spectrum of **1** has only ABX systems for the heterocyclic ring protons (H_{6a}, H_{5a} & H_{5e}) since no coupling partner is available at C_3 for C_2 proton, the benzylic proton at C_2 appeared as a singlet at 3.79 ppm. The chemical shift value of H_2 benzylic proton when compared to that of the 3-methyl analog **3** indicated the axial position for the proton and equatorial orientation for the chlorophenyl group. The signal at 4.59 ppm with ^{3}J values of 11.1 ($^3J_{6a, 5a}$) and 5.1 Hz ($^3J_{6a, 5e}$), is assigned to the axial proton at C_6 (H_6) and it confirmed the equatorial orientation of the chlorophenyl group at C_6. The coupling constant ($^3J_{6a, 5a}$ & $^3J_{6a, 5e}$) data were employed to calculate the dihedral angles between the vicinal protons (H_6 & H_{5a}, H_{5e}) by DAERM. The *cis* (H_6-C_6-C_5-H_{5e}) and trans (H_6-C_6-C_5-H_{5a}) dihedral angles of **1** were found to be **45°** & **165°** respectively. The observed vicinal coupling constants and dihedral angles confirmed that the compound **1** prefer to exist in the chair conformation. The signal at 2.75 ppm which appears as double doublet ($^2J_{5a, 5e}$ = 14.0 Hz and $^3J_{6a, 5a}$ = 12.0 Hz) can be assigned to the axial proton of C_5 (H_{5a}). Similarly the signal at 2.66 ppm appeared as a double doublet with coupling constant values of 14.0 Hz ($^2J_{5a, 5e}$) and 3.5 Hz ($^3J_{6a, 5e}$) can be assigned to the equatorial proton at C_5 (H_{5e}).

The presence of NH proton at 1.75 ppm was confirmed using the D_2O exchange studies (**Spectrum 3**).

The ^{13}C NMR spectrum signals (**Spectrum 4**) of the Compound **1** were assigned on the basis of additivity and by comparison with those of the corresponding 2,6-bis (2-clorophenyl) -3-methyl piperidin -4- one(**3**).

On the basis of the above discussion, it has been concluded that *r*-2,*c*-6-bis (2-chlorophenyl) -*c*-3,*t*-3-dimethylpiperidin-4-one (**1**) prefers to adopt a chair conformation with the equatorial orientation of chlorophenyl groups at C_2 and C_6 positions (**Fig. 1**).

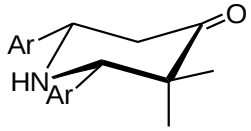
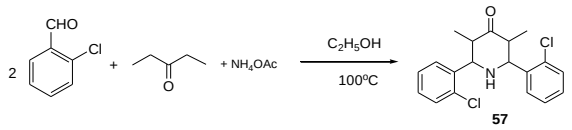


Fig. 1

The complete assignments of ^{1}H and ^{13}C NMR spectral data are presented in **Table 1 & 2**.

The piperidin-4-one **2** was synthesized by the reaction of pentan-3-one, 2-chlorobenzaldehyde and ammonium acetate in ethanol medium at 100 °C (**Scheme 2**).



Scheme 2

The structure of the compound was confirmed by IR spectra, ^{1}H, ^{13}C NMR, 2D NMR and mass spectral data.

The presence of NH stretching band (3310 cm^{-1}) and >C = O stretching band (1704 cm^{-1}) in the IR spectrum of the compound **2** indicated the formation of the compound **2**.

The compound **57** is symmetrical in nature and the assignment of ^{1}H NMR chemical shifts is very simple. The protons at C_2 and C_6 are chemically equivalent. Similarly the protons at C_3 and C_5 are also equivalent. Hence the ^{1}H NMR spectrum of **2** has only AX spin system for the heterocyclic ring protons.

The benzylic protons (H_{2a} and H_{6a}) showed a doublet at 4.38 ppm with $^{3}J_{2a,\,3a}$ (= $^{3}J_{6a,\,5a}$) value of 10.3 Hz, indicating that these two protons are diaxially oriented which in turn confirm the equatorial orientation of chlorophenyl groups at C_2 & C_6 and methyl groups at C_3 & C_5 respectively. The diaxial coupling constant of 10.3 Hz confirms the preference of chair conformation for the compound **2**.

On the basis of the above observations, it has been concluded that *r*-2,*c*-6-bis (2-chlorophenyl)-*t*-3,*t*-5-dimethylpiperidin-4-one (**2**), exist in chair conformation with the equatorial orientation of chlorophenyl substituent of C_2 and C_6 and methyl groups at C_3 and C_5 respectively similar to the previous compound .

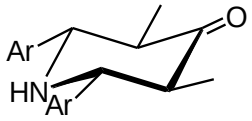
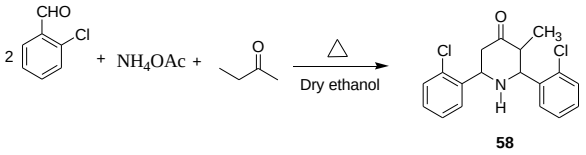


Fig. 2

The complete assignments of ^{1}H and ^{13}C NMR spectral data are presented in the **Table 3 and 4.**

3.2. r-2,c-6-Bis(2-chlorophenyl)-t-3-methylpiperidin-4-one (3)

The titled compound was synthesized by the reaction of butan-2-one,2-chlorobenzaldehyde and ammonium acetate in ethanol medium at 100 ^{o}C (**Scheme 3**).



Scheme 3

The structure of the compound was confirmed by IR spectra, ^{1}H & ^{13}C NMR spectral data. In addition DEPT spectrum was also used for the assignment of ^{13}C NMR spectrum.

The ^{1}H NMR spectrum of **3** has ABX and AX spin systems for the heterocyclic ring protons. The H_{6a}, H_{5a} and H_{5e} protons which belongs to the ABX spin system and the H_{6a} and H_{5e} protons (AX spin system) showed two double doublets at 4.07 and 2.62 ppm respectively, were assigned on the basis of the magnitudes of their coupling constant (J) values. The H_{5a} of the ABX spin system was found to have been mingled with the H_{3a} (multiplet) of the AX spin system. The signal at 4.07 ppm with ^{3}J values of 11.5 and 3.5 Hz, ascribable to $^{3}J_{6a,5a}$ and $^{3}J_{6a,5e,}$ respectively, was assigned to the axial proton at C_6 (H_6) which confirmed the equatorial orientation of the chlorophenyl group at C_6. The signal at 2.62 ppm, can also be assigned to the equatorial proton at C_5 (H_{5e}). Similarly, the proton H_2 of the AX spin system gave a doublet at 3.61 ppm with a $^{3}J_{2a,3a}$ value of 10.5Hz, indicating that these two protons are diaxially oriented, which in turn confirmed the equatorial orientation of the chlorophenyl and methyl groups at C_2 and C_3, respectively. Due to the coupling with CH_3 protons, the H_{3a} proton appeared as a multiplet at 2.62 ppm. The coupling constant ($^{3}J_{6,5a}$ & $^{3}J_{6,5e}$) data were employed to calculate the dihedral angles between the vicinal protons (H_6 & H_{5a}, H_{5e}) by DAERM.[74] The *cis* (H_6-C_6-C_5-H_{5e}) and *trans* (H_6-C_6-C_5-H_{5a}) dihedral angles of **3** were found to be **54°** and **174°**, respectively. The observed vicinal coupling constants and dihedral angles are consistent with the chair conformation for **3.**

On the basis of the above discussion, it was concluded that *r*-2,*c*-6-bis(2-chlorophenyl)-*t*-3-methylpiperidin-4-one (**3**), similar to other 2,6-diphenyl piperidin-4-ones, prefers to adopt a chair conformation with the equatorial orientation of chlorophenyl substituents at C_2 & C_6 and methyl group at C_3 respectively(**Fig. 3**).

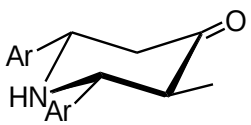


Fig. 3

The complete assignment of ^{1}H &^{13}C NMR data are presented in the **Table 5 & 6.**

4. SUMMARY

Three piperidin-4-ones *viz*. *r*-2,*c*-6-bis(2-chlorophenyl)-*c*-3,*t*-3-dimethylpiperidin-4-one **(1)**, *r*-2,*c*-6-bis(2-chlorophenyl)-*t*-3,*t*-5-dimethylpiperidin-4-one **(2)** and *r*-2,*c*-6-bis (2-chlorophenyl)-*t*-3-methylpiperidin-4-one (**3**) have been synthesized.

The preferred conformations of these compounds 1-3 have been determined using IR spectra, ^{1}H, ^{13}C, DEPT and 2D (^{1}H, ^{1}H-COSY & ^{1}H, ^{13}C-HETCOR) NMR spectra. The NMR data indicated that the parent piperidin-4-ones 56-58 adopt chair conformation.

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Table 1. Assignment of ^{1}H NMR Spectrum of *r*-2,*c*- 6- bis (2-chlorophenyl) -*c*-3, *t*-3 -dimethyl piperidin-

S. No	Chemical Shift (δ ppm)	Assignment	Coupling Constant (Hz)
1	7.39 – 7.20 (m, 8H)	Aromatic protons	
2	4.59 (dd, 1H)	H_{6a}	$J_{5a, 6a}$ = 11.1 $J_{5e, 6a}$ = 5.1
3	3.79 (s, 1H)	H_{2a}	
4	2.75 (dd, 1H)	H_{5a}	$J_{5a, 5e}$ = 14 $J_{5a, 6a}$ = 12
5	2.66 (dd, 1H)	H_{5e}	$J_{5a, 5e}$ = 14 $J_{5e, 6a}$ = 3.5
6	1.75* (s, exchangeable with D_2O)	NH	
7	1.26 (s, 3H)	CH_3 at C_3	
8	1.02 (s, 3H)	CH_3 at C_3	

* Extracted from ^{1}H NMR (D_2O exchanged) Spectrum

Table 2. Assignment of ^{13}C NMR spectrum of *r*-

S. No	Chemical Shift (δ ppm)	Assignment
1	211.74	C_4 >= O
2	140.2, 136.9, 134.2,132.5	Aromatic (*ipso*) Carbons
3	130.5, 129.6, 128.7, 128.6,127.4,126.3	Aromatic Carbons
4	63.03	C_2
5	57.09	C_6
6	50.85	C_3
7	44.68	C_5
8	20.45	CH_3 at C_3
9	20.02	CH_3 at C_3

Table 3. Assignment of ^{1}H NMR Spectrum of *r*-2,*c*-6-bis(2-chlorophenyl) *t*-3,*t*-5-dimethylpiperidin-4-one (2)

S. No	Chemical Shift (δ ppm)	Assignment	Coupling Constant (Hz)
1	7.35 – 7.19 (m, 8H)	Aromatic protons	
2	4.38 (d, 2H)	H_{2a} & H_{6a}	$J_{2a, 3a}$ = $J_{5a, 6a}$ = 10.3
3	2.79 (bs, 2H)	H_{3a} & H_{5a}	
4	1.75(bs, exchangeable with D_2O)	-NH	
5	0.92 (d, 6H)	-CH_3 at C_3 & C_5	J = 6.5

Table 4. Assignment of ^{13}C NMR Spectrum of *r*-2,*c*-6-bis(2-chlorophenyl) *t*-3,*t*-5-dimethylpiperidin-4-one (2)

S. No	Chemical Shift (δ ppm)	Assignment
1	210.14	C_4 >= O
2	139.1, 133.9	Aromatic (*ipso*) Carbons
3	129.4, 128.7,127.3	Aromatic Carbons
4	62.49	C_2 & C_6
5	51.98	C_3 & C_5
6	9.93	CH_3 at C_3 & C_5

Table 5. Assignment of ^{1}H NMR spectrum of *r-2 c*-6-bis(2-chlorophenyl)- *t-3*- ethylpiperidin-4-one (3)

S. No	Chemical shift (δ ppm)	Assignment	Coupling constant (Hz)
1	7.46 to 7.26 (8H)	Aromatic protons	
2	4.07 (dd, 1H)	H_{6a}	$J_{5a, 6a}$ = 11.5 $J_{5e, 6a}$ = 3.5
3	3.61 (d, 1H)	H_{2a}	$J_{2a, 3a}$=10.5
4	2.62 (m, 3H)	H_{3a} & $H_{5a,5e}$	
5	2.45* (s, exchangeable with D_2O)	NH	
6	0.83 (d, 3H)	CH_3 at C_3	J = 6.5

*Extracted from ^{1}H NMR (D_2O exchanged) spectrum.

Table 6. Assignment of ^{13}C NMR spectrum of *r-2,c*-6-bis(2-chlorophenyl)- *t-3*-methylpiperidin-4-one (3)

S. No	Chemical shift (δ ppm)	Assignment
1	208.61	C_4 >=O
2	141.08, 140.22, 133.60, 133.02	Aromatic (*ipso*) carbons
3	131.5, 129.0, 128.9, 128.8, 127.8	Aromatic carbons
4	67.69	-C_2
5	60.89	-C_6
6	51.63	-C_3
7	50.81*	-C_5
8	10.06	-CH_3 at C_3

*Extracted from DEPT spectrum.

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SYNTHESIS OF 3-(PYRIDYL)-3,3A,4,9-TETRAHYDRO-2*H*-PYRAZOLINO[3',4':5,4]CYCLOPENT[*B*]INDOLE

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ABSTRACTS

Mixed aldol condensation of 1-oxo-1,2,3,8-tetrahydrocyclopent[*b*]- indole (**1a-d**) with pyridine-2-aldehyde (**2**) under basic condition led to formation of 2-pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[*b*]indole (**3a-d**). To an ethanolic solution of 2-pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[*b*]indole(**3a-d**) is refluxed with hydrazine hydrate resulted 3-pyridyl-3,3a,4,9-tetrahydro-2*H*-pyrazolino[3',4':5,4]cyclopent[*b*]indole (**4a-d**).

Key words : 1-oxo-1,2,3,8-tetrahydrocyclopent[*b*]indole, pyridine-2-aldehyde, 2-pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[*b*]indole, 3- pyridyl- 3,3a, 4,9- tetrahydro- 2*H*- pyrazolino [3',4':5,4] cyclopent [*b*]indole.

1. INTRODUCTION

There has been a great deal of intrest in annelated heterocycles for designing novel structures capable of performing multiple functions. Among the numerous indole alkaloids those containing b-fused cycloalkanes (Benoit Joseph *et al.,* 2001), unit in general and b-fused cyclopentane (Elisabeth Conchon *et al.,* 2008), unit in particular are reported to possess potential pharmacological activities (Julien Debray *et al.,* 2010),. Indole is main constituent unit in many of the alkaloids of the natural origin. Indole and its derivatives are shown to exhibit antitumour, antiinflammatory, antibacterial (Sangeetha Velusamy *et al*., 2003), and antifungal (Sangeetha Velusamy *et al*., 2003), activities. Pyrazolines have also been reported to possess excellent

antibacteria (Sangeetha Velusamy *et al*., 2003), antifungal (Sangeetha Velusamy *et al*., 2003), and antiviral activities (Thomas Lemster, et. al., 2009),. These compounds owe their activities to the heterocyclic ring present in the structure. The structural and biosignificance of indoles as well as pyrazolines (Youssef Hajbi, *et al*., 2010), has infused interest in us to synthesize some unknown pyrazolino[3',4':5,4]cyclopent[b]indole derivatives (**3a-d**) utilizing 2-pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (**2a-d**) as synthons to construct pyrazolino annelated rings on the cyclopent[b]indole skeleton. The new products have been characterized by C,H,N analysis, IR, 1H NMR and mass spectral studies.

2. RESULTS AND DISCUSSION

2.1. Synthesis of 2-Pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (3a).

1-Oxo-1,2,3,8-tetrahydrocyclopent[b]indole (**1a-d**) obtained were considered to be an efficient precursor for the synthesis of many novel heterocyclo fused cyclopent[b]indoles. Mixed-aldol condensation of 5-methyl-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (**1a**) with pyridine-2-aldehyde (2) under basic condition led to the formation of 5-methyl-2-pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (**3a**) in 90% yield (**Scheme – 1**).

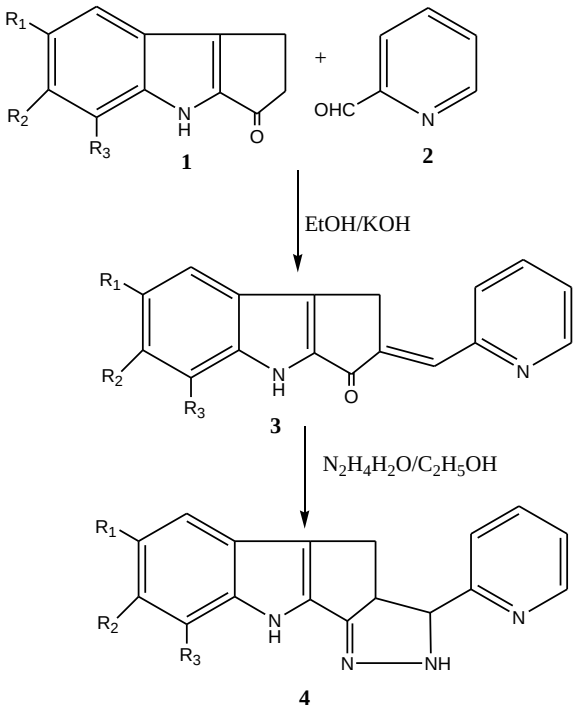
The IR spectrum of **3a** sexhibited a strong absorption bands at 1675cm-1 characteristic of α,β-unsaturated carbonyl group and at 3118 cm-1 ascribable to –NH group. The 1H-NMR spectrum showed the disappearance of C2 methylene proton signal and appearance of olefinic proton signal as a singlet at δ 7.21, which proved the mixed aldol condensation of 5-methyl-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (**1a**) with pyridine-2-aldehyde (**2**) to give 5-methyl-2-pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (**3a**). A singlet for three protons appeared at δ 2.38 was assigned to C5-CH_3 protons. The seven aromatic protons appeared as a multiplet at δ 7.34 to δ 8.74. The resonance owing to C3 methylene protons appeared as a singlet at δ 4.11, while that of –NH proton appeared as a broad singlet at δ 11.75. The molecular ion peak in its mass spectrum at m/z 275 and elemental analysis C 79.28% H 05.08% N 10.28% in accordance with the molecular formula $C_{16}H_{14}N_{20}$. A series of compounds (**2b-d**) on reaction with pyridine-2-aldehyde. The characterization data of compounds (**2a-d**) are given in the Table 1.

2.2. Synthesis of 3-(pyridyl)-3,3a,4,9-tetrahydro-2H-pyrazolino[3',4':5,4]cyclopent[b]indole (4a).

Reaction of 2-pyridyline-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (**3a**) with hydrazine hydrate in ethanol, gave 3-pyridyl-3,3a,4,9-tetrahydro-2H-pyrazolino [3',4':5,4] cyclopent [b] indole (**4a**) in 65% yield. The IR spectrum revealed

the presence of >C=N (1537 cm-1) and absence of carbonyl absorpsion. The 1H-NMR spectrum of 6-methyl-3-(pyridyl)-3,3a,4,9-tetrahydro-2H-pyrazolino[3',4':5,4]cyclopent[b]indole in CDCl3 showed a singlet three protons at 2.78 corresponding to C6-CH_3 protons. The multiplet observed in the region δ 2.47-2.88 was assigned to C3 and C3a protons. The signal due to C4 methylene protons appeared as a singlet at δ 3.70. The resonance due to pyrazolino –NH appeared as a broad singlet at δ 4.51 and indole –NH was found at δ 8.47 as a broad singlet. A multiplet appeared in the region at δ 6.92-7.58 have been assigned to aromatic protons based on their integrations corresponding seven protons respectively. The mass spectrum showed the molecular ion peak at m/z 289. The elemental analysis agreed well with the proposed molecular formula $C_{18}H_{16}N_4$. The compounds (**4b-d**) were synthesized similarly from 2-pyridyline-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (**2b-d**). The characterization data of compounds (**4a-d**) are given In the Table 2.

Scheme 1



1,3,4 **a** : $R_1 = CH_3$, $R_2 = R_3 = H$; **b** : $R_2 = CH_3$, $R_1 = R_3 = H$; **c** : $R_3 = CH_3$, $R_1 = R_2 = H$; **d** : $R_1 = R_2 = R_3 = H$

3. EXPERIMENTAL SECTION

Melting points were determined on mettler FP-5 instrument and are uncorrected. IR spectra of the new compounds have been recorded as KBr pellets on a Perkin-Elmer model 1600 FT-IR instrument in the region 4000 – 400 cm^{-1} and ^1H NMR spectra were recorded on a varian AMX-400 instrument using TMS as an internal standard. C, H, N analyses were performed on carlo erba 1108 model elemental C H N analyser. Electron impact mass spectrum was recorded using Jeol(D)-300 EI mass spectrometer.

3.1. Preparation of 2-Pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (3)

A mixture of 1-oxo-1,2,3,8-tetrahydrocyclopent[*b*]indole (**1**) (0.001 mol) and pyridine-2-aldehyde (0.001 mol) was treated with 4% alc. KOH stirred 12 hrs at room temperature. The product precipitated as crystalline solid that was filtered off and washed with 50% aq ethonal. Another quantum of the same crystalline condensation compounds was obtained from the filtrate on neutralization with acetic acid followed by dilution with water. Pure crystals are crystallized from methanol. The product was obtained were separated through colum packed with silica gel and eluting with peteroleum ether – ethyl acetate mixture [85 : 15]. The product obtained by the removal of solvent mixture offered 2-Pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[*b*]indole.

3.2. Preparation 3-(pyridyl)-3,3a,4,9-tetrahydro-2H-pyrazolino[3',4':5,4]cyclopent[b]indole (4).

To an ethanolic solution of 2-Pyridelydine-1-oxo-1,2,3,8-tetrahydrocyclopent[*b*]indole (**3**) (0.001 mol) (20 ml), hydrazine hydrate (0.5 ml) was added and mixture was refluxed. After a period of 2hrs the solvent was removed under reduced pressure and the residue was washed with water and extracted with chloroform (3 x 15 ml), and the combined organic layer was dried over anhydrous sodium sulphate. Evaporation of the solvent followed by crystallization yielded the desired 3-(pyridyl)-3,3a,4,9-tetrahydro-2*H*-pyrazolino[3',4':5,4]cyclopent[*b*]indole as yellow powder.

Table 1. Characterization of compounds 3a-d

Compd.	mp°C	Yield(%)	Mol.Formula (Mol. Wt)	Calcd % (Found)		
				C	H	N
3a	257	70	$C_{18}H_{14}N_2O$	79.39	05.14	10.21
3b	244	68	$C_{18}H_{14}N_2O$	79.28	05.08	10.28
3c	222	57	$C_{18}H_{14}N_2O$	79.18	05.02	10.26
3d	234	54	$C_{17}H_{12}N_2O$	78.30	04.58	10.67

^{1}H NMR ($CDCl_3$) (δ ppm) of compounds 3a-d

2.38 (s, 3H, C_5-CH_3), 4.11 (s, 2H, C_3-H), 7.34-8.54(m, 7H, C_4-H, C_6-H, C_7-H, C_3'-H, C_4'-H, C_5'-H, C_6'-H), 7.67 (s, 1H, olefinic – H), 11.75 (bs, 1H, carbazole H)
2.40 (s, 3H, C_6-CH_3), 4.15 (s, 2H, C_3-H), 7.34-8.54(m, 7H, C_4-H, C_5-H, C_7-H, C_3'-H, C_4'-H, C_5'-H, C_6'-H), 7.73 (s, 1H, olefinic – H), 11.69 (bs, 1H, carbazole H)
2.32 (s, 3H, C_7-CH_3), 4.25 (s, 2H, C_3-H), 7.46-8.55(m, 7H, C_4-H, C_5-H, C_6-H, C_3'-H, C_4'-H, C_5'-H, C_6'-H), 7.80 (s, 1H, olefinic – H), 11.75 (bs, 1H, carbazole H)
4.33 (s, 2H, C_3-H), 7.42-8.55(m, 7H, C_4-H, $C5_4$-H, C_6-H, C_7-H, C_3'-H, C_4'-H, C_5'-H, C_6'-H), 7.98 (s, 1H, olefinic – H), 11.77 (bs, 1H, carbazole H)

Table 2. Characterization of compounds **4a-d**

Compd	mp°C	Yield(%)	Mol.Formula (Mol. Wt)	Calcd% (Found)		
				C	H	N
4a	185	74	$C_{18}H_{16}N_4$ (288)	74.97	05.59	19.43
4b	140	68	$C_{18}H_{16}N_4$ (288)	74.88	05.57	19.36
4c	204	77	$C_{18}H_{16}N_4$ (288)	74.87	05.56	19.44
4d	198	64	$C_{18}H_{16}N_4$ (274)	74.46	05.25	20.48

^{1}H NMR ($CDCl_3$) (δ ppm) of compounds **4a-d**

2.47-2.88 (s, C_3-H, C_{3a}-H), 2.78 (s, 3H,C_6-CH_3), 3.70(m, 2H, C_4-H_2), 4.51 (s, 1H, pyrazolino-NH), 6.92-7.58(m, 7H, C_5-H,C_7-H,C_8-H, C_3'-H, C_4'-H, C_5'-H, C_6'-H), 8.47(bs, 1H, Indole –NH)
2.37-2.80 (s, C_3-H, C_{3a}-H), 2.79 (s, 3H,C_7-CH_3), 3.70(m, 2H, C_4-H_2), 4.51 (s, 1H, pyrazolino-NH), 6.92-7.58(m, 7H, C_5-H,C_6-H,C_8-H, C_3'-H, C_4'-H, C_5'-H, C_6'-H), 8.47(bs, 1H, Indole –NH)
2.47-2.88 (s, C_3-H, C_{3a}-H), 2.78 (s, 3H,C_8-CH_3), 3.70(m, 2H, C_4-H_2), 4.51 (s, 1H, pyrazolino-NH), 6.92-7.58(m, 7H, C_5-H,C_6-H,C_7-H, C_3'-H, C_4'-H, C_5'-H, C_6'-H), 8.47(bs, 1H, Indole –NH)
2.47-2.88 (s, C_3-H, C_{3a}-H), 3.70(m, 2H, C_4-H_2), 4.51 (s, 1H, pyrazolino-NH), 6.92-7.58(m, 8H, C_5-H, C_6-H, C_7-H,C_8-H, C_3'-H, C_4'-H, C_5'-H, C_6'-H), 8.47(bs, 1H, Indole –NH)

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CHEMICAL CHARACTERIZATION OF THE ACTIVE CONSTITUENTS PRESENT IN DIFFERENT FORMS OF *EMBLICA OFFICINALIS* (AMLA)

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ABSTRACT

The chemical characteristics of different forms of *Emblica officinalis* - fresh amla, sweet amla, dried amla, salted amla and lehyam - were analyzed for total phenol, vitamin C, carbohydrate, fat and total antioxidant capacity. The ash of the respective samples was used to determine the iron, calcium and phosphorus content. Higher percentage of total antioxidant capacity was observed in all the samples, which depends on the concentration of the phenolic compounds. In conclusion it can be stated that the vitamin C content (361.90mg/100g) and iron content (0.012mg/100mg) were higher in fresh form of *Emblica officinalis* whereas dried amla showed higher level of phosphorus (0.14%), phenolic acids (10%) compared to all other form of *Emblica officinalis*.

Key-words: Chemical characteristics, *Emblica officinalis*, total phenol, vitamin C.

1. INTRODUCTION

Amla (Figure 1) belonging to Euphorbiaceous family known as *Phyllanthus emblica,* Indian gooseberry or *Emblica officinalis* is a natural, efficacious, antioxidant, with the richest natural source of vitamin C. Numerous studies conducted on *Emblica officinalis* fruit suggest that it has anti-viral properties and also functions as an anti-bacterial and anti-fungal agent (Sampath Kumar *et al.,* 2012). According to ancient Indian, it is the first tree to be created in the Universe. The species is native to India and also grows in tropical and subtropical regions including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia. The fruits of *Emblica officinalis* are widely used in the Ayurveda and are believed to increase defense against diseases. It has its beneficial role in cancer, diabetics, liver treatment, heart trouble, ulcer, anemia and various other diseases (Sachan *et al.,* 2013). Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastro protective agent. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level. It is believed by ayurvedic practitioners that regular intake of amla reduces the onset of chronic diseases (Khan, 2009).

The edible fruit tissue contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple (Puri *et al.,* 1970). Fruits, whether fresh or dried, have always formed a part of the stable diet of human beings. The reason for that, they are rich in nutrients and provide some of the essential minerals, vitamins and the like, to our body. Apart from that they also help in curing a number of diseases, *Emblica officinalis* is one of the important herbal drugs used traditionally both as a medicine and as tonic to build up, lost vitality and vigor.

Figure 1. Amla - *Emblica officinalis*

No published data could be found relative to the composition of the various forms of amla, hence in the present study the chemical constituents in the different forms of *Emblica officinalis* like fresh amla, sweet amla, dried amla, salted amla and lehyam was studied.

2. MATERIALS AND METHODS

2.1. Preparation of samples

5g of fresh amla (Figure 2), sweet amla (Figure 3), dried amla (Figure 4), salted amla (Figure 5) and amla lehyam (Figure 6) were ground with 50ml of water by using motor pestle. These solutions were filtered and the filtrates were stored in different containers.

2.2. Determination of total phenols

0.1ml of each samples were taken in a different test tubes. 0.9ml of distilled water and 0.5ml of Folin-Ciocalteau reagents were added

(Singleton and Rossi, 1965). After three minutes, 2ml of 20% sodium carbonate was added to it. Likewise, the blank was also prepared. The test tubes were heated for one minute and cooled. Then, the optical densities were measured at 650nm using UV-Spectrophotometer.

Figure 2 Fresh amla **Figure 3 Sweet amla**

Figure 4 Dried amla

Figure 5 Salted amla

Figure 6 Lehyam

10, 20, 30, 40 and 50ml of standard gallic acid solutions were prepared and optical densities were noted. The graph was plotted absorbance against concentration. The concentration of phenols in the aliquot of the samples can be read directly from the calibration.

2.3. Determination of Vitamin C

10ml of each sample were taken in a separate 100ml conical flask and titrated with the dye solution. The end point was the appearance of pink colour (Sadasivam *et al.,* 1987).

$$\text{Ascorbic acid} = \frac{\text{Titre value x Dye factor x Volume made up x 10}}{\text{Aliquot of extract taken for estimation x Volume of the sample}}$$

2.4. Determination of Carbohydrates

0.1g of dried amla and 1ml of fresh amla, sweet amla, salted amla and lehyam extracts were taken in separate boiling test tubes. These samples were digested with 10ml 2.5N hydrochloric acid for three hours. After digestion, the solutions were neutralized with sodium carbonate. Then, the solutions were filtered and made up to 100ml with water. 0.5ml of samples was taken in separate test tubes. 0.9ml of distilled water and 4ml of Anthrone reagent were added to it. The blank solution was also prepared. The test tubes were boiled for 10 minutes and cooled. Then, the optical densities were measured at 630nm using UV-Spectrophotometer. 10, 20, 30, 40, 50, 60, 80, 100ml of standard glucose solutions were prepared and optical densities were noted using UV-Spectrophotometer. The graph was plotted with absorbance against concentration. The concentration of carbohydrates in the aliquot of samples can be read directly from the calibration (Hedge *et al.,* 1962)

2.5. Determination of Iron

1g of each samples were ashed in separate crucibles. The ashes were digested with 2ml of Conc. HCl, 2ml of picric acid and 2ml of Conc. HNO_3. After digestion, the solutions were diluted to 10ml with distilled water. 1ml of the each diluted solutions were taken in separate test tubes. 0.5ml of Conc. H_2SO_4, 1ml of potassium persulphate and 2ml of potassium thiosulphate were added to it and made up to 15ml with distilled water. The blank was also prepared. Then, the optical densities were measured at 480nm using UV-Spectrophotometer .

$$\text{Iron (mg/100g)} = \frac{\text{OD of sample x 0.1 x Total volume of ash solution x 100}}{\text{OD of standard x 5 x weight of sample taken for ashing}}$$

0.2, 0.4, 0.6, 0.8, 1ml of standard iron sulphate solutions was prepared and optical densities of solutions were measured. The graph was plotted with absorbance against concentration. The concentration of iron contents in the aliquot of the samples can be read directly from the graph.

2.6. Determination of Calcium

1g of each samples were ashed in separate crucibles. The ashes were digested with 2ml of Conc. HCl, 2ml of picric acid and 2ml of Conc. HNO_3. After digestion, the solutions were diluted to 10ml with distilled water. 0.5ml of the diluted solutions were taken and made up to 10ml with distilled water. The standard solution was prepared. Then, the amount of calcium present in the samples was measured by using flame photometer.

2.7. Determination of Fat

10ml of samples were refluxed with 20ml of hexane for one hour. Then, the solutions were filtered off. The filtrates were collected in separate previously weighed petri plates. Again 20ml of hexane was added to the reflux container. The solutions were refluxed and filtered. The filtrates were combined and kept in the oven. The contents of the petri plates were dried and weighed. It gave the amount of fat present in the samples (Sadasivam *et al.,* 1987).

2.8. Determination of Phosphorus

0.1ml acid digestion solutions of samples were taken in separate 100ml standard flasks and made up to 100ml with distilled water. One drop of phenolphthalein indicator was added to it. The pink was obtained. Then, dilute sulphuric acid was added in drop wise to discharge the colour. 2ml of ammonium molybdate and 0.25ml of stannous chloride were added to it. The solutions were mixed thoroughly. The blank solution was also prepared. After 10 minutes, the optical densities of the solutions were measured at 690nm using UV-Spectrophotometer.

5, 10, 15, 20, 25ml of standard potassium dihydro phosphate solutions were prepared and optical densities of the solutions were measured. The graph was plotted with absorbance against concentration. The concentration of phosphorus in the aliquot sample can be read directly from calibration.

2.9. Determination of Total antioxidant capacity

0.2ml of each sample extracts were taken in separate test tubes. 0.2ml of 2.5% linoleic acid, 0.4ml of phosphate buffer solution (pH=7) and 0.2ml distilled water were added to it. The test tubes were kept at dark in 40^0C for five hours. 0.1ml of the solutions was taken for analysis. 9.7ml of 75% ethanol and 0.1ml of 30% ammonium thiocyanate were added and left for five minutes. 0.1ml 20mM $FeCl_3$ in 3.5% HCl was added to it. The blank and control solutions were also prepared. Then, the optical densities of the samples were measured at 500nm using UV-Spectrophotometer. Total antioxidant capacity is then calculated using following formula.

$$\text{Total antioxidant capacity} = \frac{\text{Control value - OD value x 100}}{\text{Control value}}$$

3. RESULTS AND DISCUSSION

Amla is one of the most celebrated herbs in the Indian traditional medicine system, and is becoming increasingly well known for its unusually high levels of Vitamin C, which is resistant to storage and heat damage due to cooking. Its fruits have potent antioxidant activity due to the presence of tannins, vitamin C and flavonoids. It has been used as rejuvenating herb.

In the present study, the chemical constituents of fresh amla fruit were compared with dried, lehyam, salted and sweetened preserved form of amla. The parameter that essentially determine the active constituents of the fruit was analyzed and it was observed that dried *Emblica officinalis* showed higher levels of chemical constituents compared to other forms of amla. The lehyam that contain high content of amla along with other plants, showed high level of fat, which could be due to the addition of ghee and oil during preparation of chayawaprash. The fat content was similar in all other preserved forms of *Emblica officinalis.*

3.1. Total phenolic content

Total phenolic content of amla extract was determined by colorimetric method. In this method phosphotungstic-phosphomolybdenum complex obtained as a blue chromophore was measured. Total phenolic contents present in different forms of amla calculated from concentrations of aliquot solutions, fresh amla (15µg), dried amla (9µg), sweet amla (7µg), salted amla (2µg) and lehyam (14µg) showed varying concentrations of phenolic content (Graph 1). And they were having different amounts of phenolic contents in its extracts (Table 1).

In the present study total phenolic content was higher in dried amla (10%) compared to fresh amla (2.4%), sweet amla (1.23%), salted amla (0.344%) and lehyam (2.46%). Klimczak *et al.,* (2006) reported the content of total phenols in fresh orange juices to be 226.7±6.4mg/L (juice 1) and 202.7±6.8mg/L (juice 2). The protein content by Folin-Ciocalteu method was higher than the concentration obtained by HPLC method 684.2±1.0mg for juice 1 and 634.6±0.9mg of caffeic acid equivalents/L for juice 2.

The results are in good agreement with those reported in the literature (Rapisarda *et al.,* 1999; Gardner *et al.,* 2000) however there is evidence that the spectrophotometric method over estimates the poly-phenolic content as compared to the chromatographic method. This can be explained by the lack of selectivity, (Escarpa and Gonzalez, 2001), which reacts not only with phenols but also with other reducing compounds such as carotenoids, amino acids, sugar and vitamin C (Vinson *et al.,* 2001). However, this method has been shown to be a useful analytical tool for the routine analysis of polyphenols and it is widely used in many laboratories for the determination of differences among fruits and vegetables and their products.

3.2. Vitamin C

Vitamin C content of the amla extracts were calculated using dye factor. In this method ascorbic acid oxidized to give dehydroascorbic acid. Vitamin C contents present in different forms of amla represented in Table 1 indicate that vitamin C content was highest in fresh amla and lowest in salted amla.

Ranganna, (1986) reported that irrespective of the treatments and storage conditions, a continuous decrease in ascorbic acid content of amla juice was observed during storage. It was observed that after six months of storage, maximum vitamin C content (232.7mg/100ml) was observed in SO_2 treated juice stored at low temperature, followed by 195.5mg/100ml in pasteurized + SO_2 treated and 189.3mg/100ml in pasteurized amla juice stored at low temperature. In present study vitamin C content was higher in fresh amla (361.904mg/100g) compared to dried amla (222.63mg/100g), sweet amla (115.27mg/100g), salted amla lehyam (104.79mg/100g) and salted amla (40.97mg/100g). The significance of vitamin C as an important antioxidant has been well established (Miller and Rice-Evans, 1997; Rapisarda *et al.,* 1999; Gardner *et al.,* 2000). The concentration of vitamin C is a significant indicator of orange juice quality and it may serve as an indicator that all processes, which ensure a high quality of the product, have been applied in the production processes. In both juices analyzed in their study, the vitamin C content was found to be similar, 408.5±0.9mg/L and 361.5±1.8mg/L in juice 1 and 2 respectively. The author's previous study, where commercial oranges delivered by different producers were assessed, showed that they contain 150 to 440mg of vitamin C in 1L of juice.

3.3. Carbohydrates

Carbohydrate content of amla extract was determined by colorimetric method. In this method glucose is dehydrated to hydroxymethyl furfural. Carbohydrate contents present in different forms of amla calculated from concentrations of aliquot solutions. Fresh amla (10µg), dried amla (55µg), sweet amla (31µg), salted amla (8µg) and lehyam (21µg) showed wide variation in carbohydrate content (Graph 2). The results represented in Table 1 showed that the highest carbohydrate content was found in dried amla (10.4%).

Dhale, (2012) reported that reducing sugar in amla was 8.6%. In present study carbohydrate content was higher in dried amla (10.4%) compared to fresh amla (1.28%), sweet amla (0.272%), salted amla (0.068%) and lehyam (0.18%).

3.4. Iron

Iron content of amla extract was determined by colorimetric method. In this method ferric thiocyanate was obtained. The concentrations of the aliquot solutions were calculated from standard iron graph. Fresh amla (0.12µg), dried amla (0.6µg), sweet amla (0.11µg), salted amla (0.22µg) and lehyam (0.1µg) showed varying concentrations of iron content (Graph 3). Then, the iron contents of the different forms of amla were calculated (Table 1).

Mishra *et al.,* (2012) reported that iron content present in *Emblica officinalis* was 17.2ppm whereas Suriyavathana *et al.,* (2011) reported that iron content was present in *Emblica officinalis* was 0.606±0.0001mg/g. In the present study, iron content was higher in dried amla (0.06mg/100mg) compared to fresh amla (0.012mg/100mg), sweet amla (0.011mg/100mg), salted amla (0.0044mg/100mg) and lehyam (0.01mg/100mg).

3.5. Calcium

Calcium content of amla was determined by flame photometer using ash solution (Table 1) showed that the highest calcium content in salted amla (0.4%) compared to other forms of amla. Dhale, (2012) reported that calcium content in *Emblica officinalis* was 0.42%. Suriyavathana *et al.,* (2011) reported that calcium content was present in the *Emblica officinalis* was 4.804±0.0029mg/g. In present study, calcium content was higher in salted amla (0.4%) compared to fresh amla (0.3%), sweet amla (0.3%), dried amla (0.2%) and lehyam (0.2%).

3.6. Fat

Fat content of amla was determined by reflux method, varied between the different forms of

amla (Table 1). Fat content was very low in all samples compared to lehyam. Mishra *et al.,* (2012) reported that crude fat content present in *Emblica officinalis* was 2.46% whereas Sachan *et al.,* (2013) reported that fat content present in *Emblica officinalis* was 0.1%. In present study, fat content was higher in lehyam (2.6%) compared to fresh amla (0.8%), dried amla (2.2%), sweet amla (1.7%) and salted amla (0.8%).

3.7. Phosphorus

Phosphorus content of amla extract was determined by the formation of molybdenum blue complex. Phosphorus contents present in different forms of amla were calculated from concentrations of aliquot solutions. Fresh amla (3.5µg), dried amla (14µg), sweet amla (3µg), salted amla (5µg) and lehyam (4.5µg) showed varying concentrations of phosphorus content (Graph 4). Then, the phosphorus contents of the different forms of amla were calculated (Table 1).

Dhale, (2012) reported that phosphorus content present in *Emblica officinalis* was 0.04%. Suriyavathana *et al.,* (2011) reported that phosphorus content in *Emblica officinalis* was 0.716±0.0001mg/g. In present study, phosphorus content was higher in dried amla (0.14%) compared to fresh amla (0.035%), sweet amla (0.03%), salted amla (0.05%) and lehyam (0.045%).

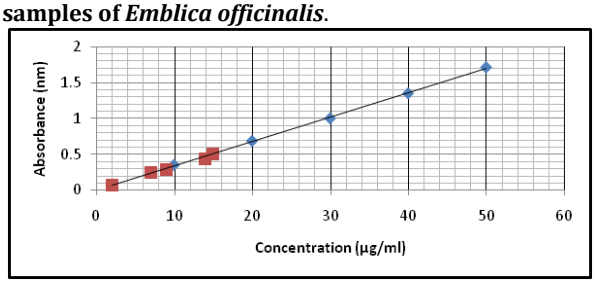
3.8. Total antioxidant capacity

Total antioxidant capacities of the different forms of amla were calculated based on the formation of oxidized compounds in relation to the control (Table 1).

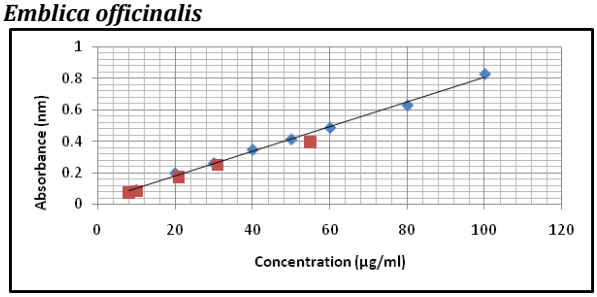
Ruangchakpe *et al.,* (2007) reported that total antioxidant capacity (µmoles TE/100g FW) in *Emblica officinalis* was 78.2 whereas Shukla *et al.,* (2009) reported that total antioxidant capacity in *Emblica officinalis* at concentration of 1mg/ml was 7.78±0.17. In present study, the total antioxidant capacity was higher in salted amla (52.55%) compared to fresh amla (49.5%), sweet amla (35.24%) and lehyam (32.58%). Maheshu *et al.,* (2011) reported that the ferric thiocyanate method determines the antioxidant activity with the measurement of the amount of peroxides formed in a linoleic acid emulsion of antioxidant, during incubation (Erkan *et al.,* 2008). The inhibitory effect of the extracts from Dolichos lablab raw and processed seed samples on the peroxidation of linoleic acid at concentration of 250µg/ml, in comparison to BHT was measured using the ferric thiocyanate method. Each extracts showed strong antioxidant activity in inhibition of linoleic acid peroxidation. From the ferric thiocyanate results, the inhibition of peroxidation in linoleic acid system of raw, dry heated and pressure cooked samples were found to be 97.2±1.02%, 98.4±1.48% and 95.6±0.98%.

Antioxidant activities are known to increase proportionality to the polyphenol content. This activity is believed to be mainly due to their redox properties (Adedapo *et al.,* 2008), which plays an important role in (a) adsorbing and neutralizing free radicals, (b) quenching singlet and triplet oxygen and (c) decomposing peroxides (Wang *et al* 1998). Also according to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. Naturally occurring antioxidants such as phenols, flavonoids are well known to have less or no side effects and hence are considered to safe (Asgarirad *et al.,* 2010).

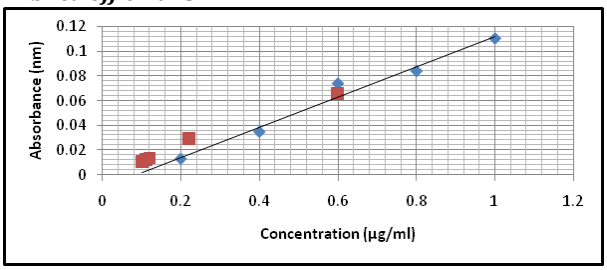
Graph 1. Standard graph of gallic acid used for the determination of total phenolic content in the test samples of *Emblica officinalis*.



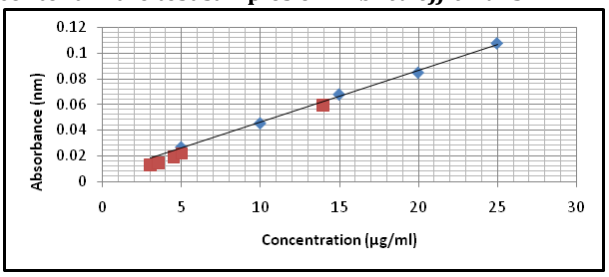
Graph 2. Standard graph of glucose used for the determination of carbohydrates in the test samples of *Emblica officinalis*



Graph 3. Standard graph of iron sulphate used for the determination iron content in the test samples of *Emblica officinalis*



Graph 4 Standard graph of potassium dihydro phosphate used for the determination of phosphorus content in the test samples of *Emblica officinalis*



4. CONCLUSION

The present study was carried out to assess the variations in the chemical characteristics of different forms of *Emblica officinalis*.

- All the samples were observed to be a good source of vitamin C and also antioxidant capacity.
- A correlation between phenolic compounds and antioxidant capacity was observed, and it can be concluded that the phenolic compounds contributes directly to antioxidant action.
- The vitamin C content (361.90mg/100g) was higher in fresh amla compared to other forms of *Emblica officinalis.*
- Fresh amla was showed high levels of total antioxidant capacity and vitamin C but lower levels of carbohydrate, fat and phosphorus content.
- Dried amla was high on total phenolic content, carbohydrate, iron, fat and phosphorus but showed low levels of vitamin C and iron compared to other forms of *Emblica officinalis*.
- All samples are found to have similar calcium content.
- Salted amla was high only on total antioxidant capacity (52.55%) but showed low levels of all other parameters
- Fresh amla and lehyam having similar total phenolic content and iron content but differ in other parameters.

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Table 1. Various chemical constituents in different forms of *Emblica officinalis*

Chemical Constituents	Fresh amla	Dried amla	Sweet amla	Salted amla	Lehyam
Total phenols	2.40	10.00	1.23	0.34	2.46
Vitamin C (mg/100g)	361.90	222.63	115.27	40.97	104.79
Carbohydrate (%)	1.28	10.40	0.27	0.06	0.18
Iron (mg/100mg)	0.012	0.060	0.011	0.004	0.010
Calcium (%)	0.30	0.20	0.30	0.40	0.20
Fat (%)	0.80	2.20	1.70	0.80	2.60
Phosphorus (%)	0.03	0.14	0.03	0.05	0.04
Total antioxidant capacity (%)	49.50	46.78	35.24	52.55	32.58

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BIOPHARMACOLOGICAL EFFECTS OF EXTRACTS OF SOME COMMONLY AVAILABLE INDIAN PLANTS ON *CHANNA STRIATA*

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ABSTRACT

Six commonly available Indian plants extracts on *Channa striata* indicate that acetylcholine synthesis and acetylcholinesterase inhibition were observed in the heart, brain, muscle and liver tissues. The possibility of using the toxic substances as fish bait is discussed.

Key words : Acetylcholine, Acetylcholinesterase, *Channa striata*.

1. INTRODUCTION

Many Indian plants have been increasingly tested for a wide variety of biological activities including antifertility, anticancer, chemotherapeutic and pharmacological activities (Agarwal and Rangari. 2003: Bol Kent *et al*., 2004).However, most of these studies were done only on mammals.

Though various plant extract are used as arrow poisons and fish baits,no study to date has evaluated the biopharmocological properties of these plant extracts on fresh water food fishes. In order to identify a substance which can be easily obtained and readily used for fish hatching purposes.We have evaluated to pharmacological effects of six commonly available poisonous plants in our area.

2. MATERIALS AND METHODS

2.1. Collection of Plants

The following plants are collected and tested.

Plant	Family	Parts Used
Datura metel	Solanaceae	Seeds
Gloriosa superb	Liliaceae	Roots
Vinca rosea	Apocynaceae	Leaves
Calotropis gigantean	Asclepiadaceae	Leaves
Antiarchis toxicaria	Moraceae	Leaves
Parthenium hysterophorus	Compositae	Leaves

2.2. Procurement of fingerlings

These plants were screened for pharmacological effects in the commonly available and economically important freshwater food fish *Channa Striata*. The fishes (10-25g) were procured from local freshwater sources and held in laboratory in large plastic tanks (02: 6-7 mg/I; pH 6.7-6.9; fish density 8-15 g/I water) for 15 days before using in the experiments.Fishes were subjected to 50% ethanolic extracts for 30 days.The level of plant extract was kept constant by changing the water everyday and adding the requistic amount of plant extract stock solution.They were fed daily prior to change of water to prevent ingestion of plant extract through food. A control group exposed to ethanol alone was also maintained. At the end of 30 day period, the fish were killed by decapitation and the tissues rapidly excised. The tissues were raised,blotted and homogenized in a motor driven all glass homogenizer with two volumes of chilled saline (0.7% Nacl). Homogenates were centrifuged at 10,000 g for 15 min.The supernatant fraction were diluted with ten volumes of chilled saline and used as the enzyme source.

2.3. Determination of Biopharmacological activity

The activities of acetylcholinesterase were determined according to the methods of Bock endahl and Ammon (1955) using 4.5 x 10-2 M acetylcholine as substrate.The acetylcholine content was determined after Metcalf (1951) and proteins by the method of Lowry *et al*. (1951). The different sets of data were examined for significant difference ($P<0.05$) by Wilcoxans two-sample test (Hodges and Lehman, 1970).

3. RESULTS AND DISCUSSION

3.1. Inhibition of Biopharmacological activity

The results are presented in the Table 1 and Table 2. From the results it is clear that acetylcholine in accumulated significantly and acetylcholinesterase is inhibited in the heart, brain, muscle and liver tissues of *C.striata*. Maximum inhibition is recorded for Parthenium and Datura. The accumulation of acetylcholine indicate the blockage of nerve impulse transmission. The rapid depletion of acetylcholinesterase again show the disruption of neuromuscular transmission.

3.2. Types of toxins in plants

All the six plants extracts contain potent toxic substances and inhibit the functioning of the nervous system.According to Viswanathan and Joshi (1983), some of the toxic substances present in the tested plants are

Datura	-	tropane alkaloids-atropine,hyoscyamine and scopolamine
Gloriosa	-	Colchicine(alkaloid)
Vinca	-	75 alkaloids (Taylor and Fransworth, 1975)
Calotropis	-	Steroidal glycoside-Calotropin
Antiaris	-	glycoside α – antiarin
Parthenium	-	sequiterpene lactone parthenin

Plant derived substances are mainly evaluated for anti-inflammatory and antifertility activities in mammals (Agarwal and Rangari, 2003; Gupta and Sharma,2003). However,recently a potent cardiotoxic factor was isolated from the skin of C.Striata (Karmakar *et al*., 2002). These bioactive compounds inhibit respiratory enzymes (Al-Hassan *et al*., 1985). The nanotoxic effect and isolation of these compounds to use as fish bait is hither to unnoticed in many plants.Pond, river and lake fishing require cheap and safe fish baits.Synthetic pesticides and insecticides not only poison the fishes baited but also pollute the aquatic environment. Bioactive compounds are safe and environmental pollution is almost nil (Bol Kent *et al*., 2004). All the six plants tested are available throughout India and in many areas they are considered to be dangerous weeds for agriculture and livestock grazing.

4. CONCLUSION

The toxic substances present in the six plants inhibit the neuromuscular transmission.These toxic substances accumulate in the fish metabolism and cause neural problems to the people those who consume it. Therefore, proper arrangements have to be taken by the government and volunteers to eradicate the unwanted weeds from the crops and agriculture field.

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Table 1.Effects of Plant Extracts on the Acetylcholinesterase and Acetylcholine Activities in the Heart and Brain of *C.Striata*

	Heart				Brain			
	AchE		Ach		AchE		Ach	
	Control	Exp	Control	Exp	Control	Exp	Control	Exp
Datena	37.41±2.88	24.50±3.11	24.51±2.76	68.90±3.81	76.12±5.01	21.41±4.80	28.63±3.40	79.52±4.06
	-34.51		-181.11		-71.19		+177.75	
Gloriosa	37.41±2.88	27.50±2.61	24.51±2.76	65.19±3.06	76.12±5.01	23.82±2.19	28.63±3.40	66.41±3.00
	-26.49		+165.97		-68.71		+131.96	
Vinca	37.41±2.88	22.41±0.89	24.51±2.76	70.65±4.16	76.12±5.01	20.43±3.01	28.63±3.40	82.40±3.93
	-29.40		+157.77		-72.53		+135.91	
Calotropis	37.41±2.88	22.41±0.89	24.51±2.76	70.65±4.16	76.12±5.01	20.43±3.81	28.63±3.40	82.40±3.93
	-40.10		+188.25		+70.16		+181.81	
Antiarcis	37.41±2.88	24.67±0.89	24.51±2.76	67.51±3.08	76.12±5.01	22.41±3.01	28.63±3.40	78.43±4.00
	-34.06		+175.44		-70.56		+173.43	
Parthenium	37.41±2.88	20.61±2.90	24.51±2.76	72.36±4.08	76.12±5.01	20.00±1.90	28.63±3.40	82.19±42.1
	-44.19		+195.23		-73.73		+187.08	

Value expressed as mean ± SD of 6 observations are significant p<0.001

Table 2. Effects of Plant Extracts on the Acetylcholinesterase and Acetylcholine Activities in the Muscle and Liver of *C.Striata*

	Muscle				Liver			
	AchE		Ach		AchE		Ach	
	Control	Exp	Control	Exp	Control	Exp	Control	Exp
Datena	36.41±2.80	19.30±3.01	21.56±2.76	67.51±3.00	26.06±2.10	14.50±1.99	13.61±1.04	25.18±1.77
	-46.99		+213.13		-44.36		-85.01	
Gloriosa	36.41±2.80	21.18±1.99	21.56±2.76	66.51±2.66	26.06±2.10	16.41±2.00	13.61±1.04	26.02±1.94
	-41.83		+208.49		-377.03		+91.18	
Vinca	36.41±2.80	20.51±1.97	21.56±2.76	68.06±3.04	26.06±2.10	16.90±2.71	13.61±1.04	23.40±2.00
	-39.14		+161.60		-41.75		+64.58	
Calotropis	36.41±2.80	20.51±1.97	21.56±2.76	68.06±3.04	26.06±2.10	16.09±2.71	13.61±1.04	23.40±2.00
	-43.55		+215.68		-35.15		+71.93	
Antiarcis	36.41±2.80	21.16±2.06	21.56±2.76	66.02±3.81	26.06±2.10	14.81±1.72	13.61±1.04	24.01±2.01
	-41.88		+206.21		-43.17		+76.41	
Parthenium	36.41±2.80	16.41±2.00	21.56±2.76	68.19±3.83	26.06±2.10	13.60±0.99	13.61±1.04	26.14±2.01
	-44.69		+216.28		-47.81		+92.06	

Value expressed as mean ± SD of 6 observations are significant p<0.001

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ETHNO-ECOLOGICAL STUDIES ON THE MEDICINAL PLANTS OF WESTERN GHATS REGION WITH SPECIAL REFERENCE TO VALPARAI TRIBES

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ABSTRACT

Traditional knowledge on plant has a long-standing history in many indigenous communities, and continues to provide useful tools for treating various diseases. Tribal communities living in biodiversity rich areas possess a wealth of knowledge on the utilization and conservation of food and medicinal plants. They are well versed in the usage of plant for treating various diseases. The present study carryout by survey method aimed to identifying the plants used for the general health of the tribal communities such as Kadar, Muthuvar and Malai Malasars of Western Ghats region. Ethno medicinal information was gathered through questionnaire from the majority of tribal people of Valparai hills Coimbatore, Tamil Nadu. All the traditional and other knowledge related to the collection and consumption of the medicinal plants, their environment on which communities depends was documented. The present study observed that, the tribal peoples from valparai having knowledge of 29 species on the traditional medicine. These tribes are one of the major conservators of environments. Their traditional knowledge can be utilized for the breeding technology of variety of threatened species and develop for the biodiversity conservation as well as for pharmacological research in various dimensions.

Keywords: Valparai tribes, Traditional knowledge, Ecological studies, Medicinal plants.

1. INTRODUCTION

India is proud to be rich in biodiversity possess about 8% of the estimated biodiversity of the world with around 12600 species. It is one of the 12 mega biodiversity centers with 2 hot spots of biodiversity in the Western Ghats and North-eastern region. It's also rich in ethnic diversity, there are about 67.37 million tribal people belonging to 537 tribal groups living in different geographical locations with various subsistence patterns (Amuthavalluvan 2011, Shanmugam *et al*., 2012).These tribal groups living in diversity rich areas possess a wealth of knowledge and skills on the utilization and conservation of food and medicinal plants. Ethnobotany is the scientific study of the relationships that exists between people and plants. Since the beginning of civilization, people have used plants as medicine. The World Health Organization (WHO, 2005) has estimated that 80% of the populations of developing countries still rely on traditional medicines, mostly plant drugs, for their primary health care needs. Demand for medicinal plant is increasing in both developing and developed countries due to growing needs of natural products being non-toxic and consider of no side-effects, apart from availability at affordable prices. The medicinal plant sector has traditionally occupied a pivotal position in the socio cultural, spiritual and medicinal areas of rural and tribal families. It is estimated that tribal people of Tamil Nadu occupy 1.05% of the total state population and 0.77% of the total tribal population of the country. Ministry of Tribal affairs has released a list of tribal communities in India for each state and Tamil Nadu contains 36 types of tribal communities and they are distributed in different districts in the forests and adjoining areas.

The practices of traditional medicine are based on hundreds of years of belief and observations, which predate the development and spread of modern medicine (Aburjai *et al.,* 2007). In developing countries, there is an increasing attempt to incorporate traditional medicines, especially herbal preparations in the local health care systems and a modernize preparations in the local health care systems and a modernized people are increasingly turning to herbal medicine (Njoroge and Wondimu *et al*., 2007). In India, medicinal plants are widely used by all sections of the population with an estimated 7500 species of plants used by several ethnic communities and it is known that India has the second largest tribal population in the world after Africa (Kala 2005). With enormously diversified ethnic groups and rich biological resources, India represents one of the great emporia of ethnobotanical wealth. Even today, tribal communities in India still collect and preserve locally available wild and cultivated plant species and

practice herbal medicine to treat a variety of diseases and disorders (Mahishi *et al*.,2005).

2 .MATERIALS AND METHODS

2.1. Study area

The Western Ghats are globally recognized for their biological diversity and extend along the west coast of India from the River Tapti in the north almost to the southern tip of the peninsula. Toward its southern ranges lie the Anamalai hills ('elephant hills' in Tamil), an important conservation area in the southern Western Ghats. The present study was carried out in Indira Gandhi Wildlife Sanctuary (earlier known as the Anamalai Wildlife Sanctuary, 987 km^2, 10° 12' N to 10° 35' N and 76° 49' E to 77° 24' E) particularly Valparai plateau fringed largely by tea estates. The altitude within the sanctuary ranges from 220 m in the foothills along the northern fringes to 2,513 m in the Grass Hills at the southern portion of the reserve. (Chandi, 2008). These hill ranges have been home to indigenous communities of different ethnic origin such as the Kadar, Muthuvar and Malai Malasars. The study was conducted in three types of tribal communities to ascertain the detailed information on the traditional healing potential of tribes inhabit the forest areas in Valparai , Coimbatore district of Tamil Nadu, South India.

2.2. Methods

The Ethnobotanical data were collected from December 2012 to November 2013 according to the methodology suggested by Jain, 2001. The ethnomedicinal data (local name, mode of preparation, medicinal uses) were collected through questionnaire, interviews and discussions among the tribal practitioners in their local language. Our questionnaire allowed descriptive responses on the plant prescribed, such as part of the plant used, medicinal uses, detailed information about mode of preparation (i.e., decoction, paste, powder and juice), and form of usage either fresh or dried and mixtures of other plants used as ingredients. They were selected based on their knowledge of medicinal plants either for self-medication or for treating others.

The species mentioned by the informants were taxonomically identified.

3. RESULTS AND DISCUSSION

The result shows that the tribal communities of Valparai especially Kadar, Muthuvar and Malai Malasars possess a very good knowledge of medicinal plants available in the forest area. The study includes information on 29 plant species belonging to 18 families (Table 1).They are used to treat liver and stomach diseases, snake bite, piles, skin diseases, hair problems, appetizer, antiseptic, gonorrhea, urinary infections, fever, cough, wound healing, anti diabetic etc. Many species of the family Amaranthaceae, Moraceae, Solanacea, Tiliacea are frequently used.

Valparai tribal practitioners use specific plant parts and specific dosages for the treatment of ailments. The plant products are consumed raw or in the form of a decoction, as infusion for oral treatment and as burnt product, ointments or raw paste when applied externally. The parts of the plants mostly used for medicinal purposes are leaves, root, stem, fruits, and the whole plant, barks (root and stem) and flowers (including the flowering heads). (Fig 2& 3). The most common forms of preparing the medicines from the plants are fresh juice, powder, paste, and decoction. Internal uses invariably predominate over external uses. Juice (almost mixed with water and goat's or cow's milk) and paste are the main methods of preparation, either for oral or for external administration. For topical use, the most important methods used are direct application of the paste or ointment (with oil). Among the different plant parts used by the tribal communities of Valparai, leaves constituted the major portion of the medicine. These indigenous methods of treatment based on medicinal plants are still an important part of their life.

All ethno medicinal plants documented in the presence study have continuously been used and also revealed that some of them are less known and some of them supplements the available earlier data. Based on their experience and common sense, the Valparai tribal communities (Kadar, Muthuvar and Malai Malasars) have the capability to search for number of uses of plants at the same time they have also the talent to exploit the plants of even a new area where they have settled. The study indicated that, the study area was rich in medicinal plants and provides evidence that medicinal plants continue to play an important role in the healthcare system of this tribal community. Therefore it is an urgent need for the scientific awareness about the importance of biodiversity and medicinal plants for the sustainable utilization of natural resources.

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Table 1: Medicinal plants and their product consumed by Tribal peoples of Valparai-Western Ghats

S.No.	Binominal/Common name	Family	Habit	Part (s) used	Diseases
1	*Alternanthera* sessilis DC/Ponnakanni	Amaranthaceae	Herb	Seed	As Contraceptive, Hair tonic & Leucoderma
2	*Achyranthus aspera* L./Nayurivi	Amaranthaceae	Herb	Leaves	Wound healing, Dog bites.
3	*Amaranhus spinosus*/Mullukerai	Amaranthaceae	Herb	leaves	Reduses urination in urinary duct
4	*Boerhavia diffusa* L./Mukkarattai	Nyctaginaceae	Herb	Leaves	Diuretic
5	*Alangium salvifolium* Wang./Azhinzal	Alangiaceae	Shrub	Stem Bark,	Stomach Problems, Jaundice, Snake bite.
6	*Cissus quadrangularis* L./Pirandai	Vitaceae	Climber	Stem & Leaves	Bone- -breakage, appetizer.
7	*Ficus bengalensis* L./Aal	Moraceae	Tree	Stem Bark,	Wound healing.
8	*Lantana indica* Roxb./ Unnichedi	Verbenaceae	Shrub	Leaves	Anti Inflammatory, Antiseptic
9	*Syzygium* sp/ Naval	Mrytaceae	Tree	Bark Seeds	Anti Diabetic
10	*Artocarpus* sp./Cheeni pala	Moraceae	Tree	Bark and seed	Liver and stomach diseases
11	*Cassia tora* /Thakara	Caesalpiniaceae	Herb	Leaves roots & seeds	Gonorrhea
12	*Cymbopogon citratus* Stapf./lemon grass	Poaceae	Herb	leaves	Body pain
13	*Coccinia grandis* (L)Voigt./kovai kai	Cucurbitaceae	Climber	leaves	Piles and Skin diseases
14	*Centella asiatica* Urb/Vallarai	Apiaceae	Herb	leaves	Memory power
15	*Solanum surattense* burm.f./Kantankathari	Solanaceae	Herb	leaves	Cough and asthma and fever
16	*S. torvum* Sw./Sundai	Solanaceae	Herb	leaves	Skin diseases
17	*Carisa spinarum* L./kalakai	Apocynaceae	Shrub	fruit	Wound healing

8	*F.glomerata* Roxb./Athi	Moraceae	Tree	Bark and fruit	Insect bite and skin diseases
19	*Grewia hirsuta* Vahl./Sirukadalai	Tiliacea	Shrub	roots	Treatment for swellings
20	*G. tiliaefolia* vahl/Sadachi	Tiliacea	Tree	fruit	Stomach problem and skin diseases
21	*G. villosa* Wild/Perukadalai	Tiliacea	Shrub	fruit	skin diseases and intestinal problem, antibiotic
22	*S. gardneri* thw./Neer naval	Mrytaceae	Tree	Bark Seeds	Anti Diabetic
23	*Ziziiphus mauritiana* Lam/Elanthai	Rhamnaceae	Tree	leaves	Skin diseases and hair treatment
24	*Terminalia bellirica* Roxb.	Combretaceae	Tree	fruit	Stomach problem
25	*Ficus religiosa*/athi	Moraceae	Tree	Bark and fruit	Decoction - Gonorrhea
26	*Barleria prionitis*/ kattukanagambaram	Acanthaceae	Shrub	leaves	Headache
27	*Solanum nigrun* L/sukutti keerai	Solanacea	Herb	leaves	Stomach problem
28	*Hibiscus rosasinesis* /Semparuthi	Malvaceae	Shrub	Leaves and flowers	Hair tonic
29	*Curcuma aromatica*/Kasturi manjal	Zingiberaceae	Shrub	Rhizome	Pimple

Figure 1: Percentage of plant parts used for the preparation in Different Category

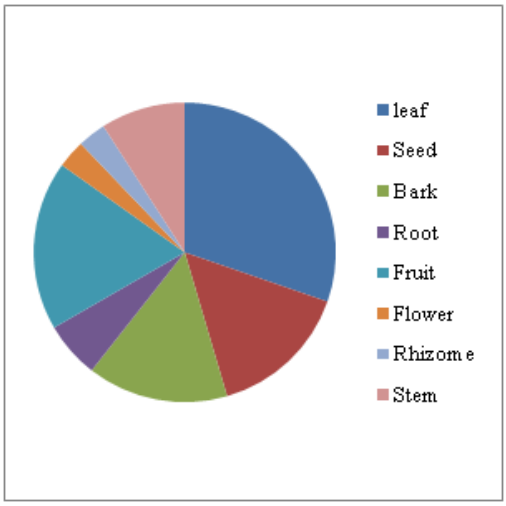
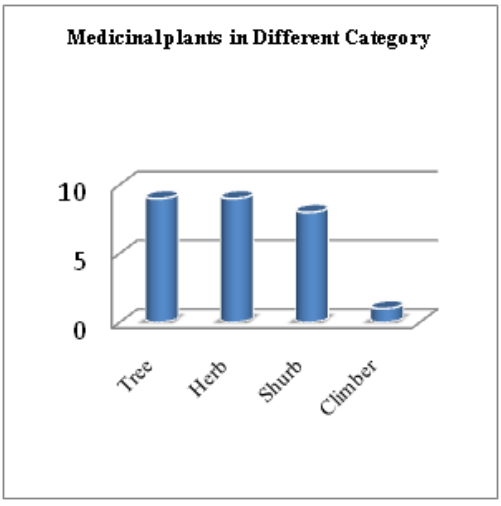


Figure 2: Medicinal plants of medicine by tribal people, Valparai region.



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PHYSICO-CHEMICAL CHARACTERISTICS OF THE PONDS CHERUKULANGARA AND ARYANAMBI IN KERALA

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ABSTRACTS

The quality of water is identified in terms of its physical, chemical and biological parameters. Limnological features of two such temple ponds, the Cherukulangara temple pond and Aryanambi temple pond were undertaken in the present study. The biological parameters of the ponds were also made during the study. In the present investigation all the parameters showed higher values in Pond B compared to Pond A. All values are found to be within or less than the permissible limit. Both ponds showed an oligotrophic nature with the presence of poor nutrients and low vegetation. Both ponds are unpolluted fresh water bodies which have no chemical or sewage pollution. It is suggested that the higher authorities should take steps to maintain the quality of fresh water bodies including ponds from all kinds of pollution and balance the fresh water ecosystem.

Keywords: Pollution,Cherukulangara temple pond,Aryanambi temple pond,Water quality and Air.

1. INTRODUCTION

Water also performs unique and indispensible activities in earth ecosystem, biosphere and biogeochemical cycles.Approximately 70% of the water in India has become polluted due to the discharge of domestic sewage and industrial effluents into natural water source, such as rivers, streams as well as lakes (Sangu and Sharma,1987).

The quality of water is described by its physical, chemical and biological characteristics. But if, some correlations were possible among these parameters, then significant ones would be fairly useful to indicate the quality of water (Dhembare *et al*., 1997). The deterioration of quality, loss of biodiversity and fast depletion of water resources are the main challenges, which need urgent attention.A pond is an earthern container for storing water. A pond os a dynamic and ever-changing community of plants and animals.

2. MATERIALS AND METHODS

The study of Cherukulangara temple was in the Palakkad district in Kerala. is situated near rice field and agricultural land, Thrichur in Kerala. The pond is used for bathing and it is a main source for the irrigation purposes during summer season. The Aryanambi temple pond is situated in Palakkad., Kerala. There are four bathing ghats in the pond.The study was conducted during the period from November to February (2012-2013) by which temperature range was in between 25^{o}C and 19^{oC}and the rainfall is about 3.5 to 2111.4mm. The analysis of all parameters like "total solids, dissolved oxygen, free carbon dioxide, carbonate, bicarbonate, calcium, magnesium, chloride, sulphate, phosphate, nitrate, iron, silicate and biological oxygen demand were estimated in the laboratory following the standard methods of APHA (2005).Qualitative analysis of plankton and fishes were also made in both the ponds A and B. Plankton were studied under compound microscope and identified with the help of standard references (Adoni *et al*., 1985; Agarker *et al*., 1994).

3. RESULTS AND DISCUSSION

During experimental period (November 2012 to February 2013), the environmental temperature ranged between 25°C and 32°C in pond A and in pond B, it was between 26°C and 33°C in pond B.The highest air and water temperature value (33°C and 25°C) was recorded in February in pond B and the lowest air and water temperature value (25°C and 19°C) was recorded in pond A in December. The water temperature was consistently lower than the atmospheric temperature. Similarly the bottom water temperature was consistently lower than the surface water.

pH values varied between 6.33 to8.0 in both A and B ponds. The maximum pH value was recorded 8.0 ± 0 in Pond B during December and minimum value was recorded 6.33 ± 0.29 in Pond A during November.In the present investigation the pH values were maximum during December and minimum during November. Besides the Pond B showing high pH value by comparing with Pond A, while both ponds keeping same variation of pH from slightly acidic to slightly alkali during November to February.

Maximum value of total solids was observed in Pond B(2333.33±288.67mg/L) and minimum value was observed in Pond A(833.33±288.67mg/L). Based on the seasonal variation it was observed that the maximum value was found during November and ranged from 1833.33mg/L to 2333.33mg/L and minimum value was observed during January and ranged from 833.33 to 1500mg/L.From these two Ponds Pond A showed high TS content than Pond B.In both the ponds,bottom water showed high TS content than surface water.

Dissolved oxygen (DO) values ranged from 3.66 mg/L to 6.34 mg/L of which maximum value (6.34± 0.28 mg/L) was noted in Pond A during December and minimum value (3.66 ± 0.05 mg/L) in Pond B during February. Seasonally it is observed that DO values was more in December followed by January, November and less value was found in February in both the ponds. From these two ponds Pond A showed high oxygen level than Pond B.In both the ponds,surface water showed high oxygen level than bottom water.

Free Carbon dioxide is also one of the most important factors in aquatic habitat. It is highly soluble in water and is the main source of carbon path way in the nature. Plant absorbs the free carbon dioxide present in both atmosphere and water. Carbon dioxide in water bodies is contributed by the respiratory activity of the animals (Vasumathy *et al*.,2009). Free carbon dioxide in the present study varied from an average of 2.75 mg/L to 14.41mg/L. The lowest value (2.75 ± 0.421 mg/L) of free carbon dioxide was recorded in the surface water of Pond A in December month (winter season) where as the highest value (14.41 ± 0.751 mg/L) was observed in the bottom water of Pond B during the period of January (pre summer season). Pond B showed consistently higher carbon dioxide than that of Pond A. Similarly the bottom water showed higher carbon dioxide than surface water. Seasonally carbon dioxide was more in January followed by November,January and less value was found in December in both ponds.

Carbonate is an important parameter which contributes to alkalinity. But in the present study, the bicarbonate was found to be absent. Bicarbonate is an important parameter which contributes to alkalinity.Value was varied from an average of 0 to 23.33mg/L 0f which maximum value(23.33± 2.886mg/L)was observed in bottom water of Pond B during February,2013(pre summer season).During December,2012 (winter season)the bicarbonate was totally absent in Pond A.

Calcium was found higher (28.33 ± 2.886 mg/L) in the bottom water of Pond B in January and lower (8.33 ± 2.886 mg/L) in the surface water of Pond A in November. The calcium value was fluctuated from an average of 11.66 to 28.33 mg/L in Pond B and is higher when it is compared with Pond A. Seasonally calcium was more in January followed by October, December and less value was found in November in both ponds. The presence of calcium in the bottom water was consistently higher than the surface water in both ponds.

Salts of magnesium are found dissolved in all water. Rocks are the main source of magnesium. It is needed by all animals for phosphate transfer involving ATP and ADP. Magnesium was found maximum (23.33 ± 2.886 mg/L) in the deeper part of Pond B in November and minimum (3.33 ± 2.886) in the surface water of Pond A in January. The magnesium value was fluctuated from an average of 8.33 ± 2.886 mg/L to 21.66 ± 5.773mg/L in Pond B is higher when it is compared with Pond A. The magnesium level in the deeper water was consistently higher than that of surface water.

The ecological significance of chloride lies in its potential to regulate salinity of water and exert consequent osmotic stress on biotic communities (Shinde *et al*., 2001). Chloride showed high significant positive relationship with water temperature, bicarbonate and calcium. The chloride content in studied ponds varied from an average of 28.4 to 68.63 mg/L in Pond A, while in the Pond B its contents ranged from 35.5 to 85.2 mg/L respectively. The chloride content was lower than the maximum permissible limit prescribed by WHO standards (1993). It was observed that Pond B having more chloride content than Pond A, where as the bottom water having high chloride content than the surface water in both ponds during the study period. Seasonally, chloride was more in February followed by November, January and less value was found in December in both ponds.

Sulphate level was maximum (98.16 ± 1.44 mg/L) in November at the bottom water of Pond B, while the minimum value (60.72 ± 1.639 mg/L) was observed in surface water of Pond A during pre summer season (February).Seasonally it can be said that sulphate was more in November followed by December, January and less value was found in February in both ponds. Pond B was leading in the amount of sulphate than Pond A in all seasons. Bottom water was observed consistently higher sulphate than surface water. The overall value was less than maximum permissible limits (500 mg/L) according to WHO standards (1993), indicating that

the pond was free from sulphate pollution during the study periods.

Phosphate level was maximum (0.84±0.15mg/L)was observed in the surface water of Pond A during February month(pre summer season). Seasonally it can be say that phosphate was more in November followed by December,January and less value was found during February in both ponds.Pond B recorded high amount of phosphate than Pond A in all seasons.Bottom water consistently higher phosphate than surface water.

Nitrate level was maximum (37.54±0.88mg/L)in February at the bottom water of Pond B, while the minimum value (18.80±1.20mg/L) in February at the surface water of Pond A.Nitrate values were more in November followed by December, January and less value was found in February in both ponds.From these two ponds Pond B showed high nitrogen level than Pond A in all seasons. Similarly bottom water showed high nitrate level than surface water.

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Table.1. Temperature of Cherukulangara temple pond during the study period Nov. 2012 – Feb. 2013.

Parameters	Nov.2012			Dec.2012			Jan.2013			Feb.2013		
	Cherukulangara Temple pond			Cherukulangara Temple pond			Cherukulangara temple pond			Cherukulangara temple pond		
	Air	Surface Water	Bottom water	Air	Surface water	Bottom water	Air	Surface water	Bottom water	Air	Surface water	Bottom water
Temperature (^oc)	28	21	19	25	19	17	26	20	18	32	24	22

Table.2. Temperature of aryanambi temple pond during the study period Nov.2012-Feb.2013

Parameters	Nov.2012 aryanambi temple pond			Dec.2012 Aryanambi temple pond			Jan.2013 Aryanambi temple pond			Feb.2013 Aryanambi temple pond		
	Air	Surface Water	Bottom water	Air	Surface water	Bottom water	Air	Surface water	Bottom water	Air	Surface water	Bottom water
Temperature (^oc)	29	22	20	26	20	18	27	21	19	33	25	23

Table.3. Chemical parameters of Aryanambi temple pond during the study period Nov.2012-Feb.2013

Parameters	Nov.2012		Dec.2012		Jan.2013		Feb.2013	
	Cherukulangara Temple pond		Cherukulangara Temple pond		Cherukulangara temple pond		Cherukulangara temple pond	
	Surface Water	Bottom water	Surface water	Bottom water	Surface water	Bottom water	Surface water	Bottom water
Total solids	1833.33±288.67	2000±500	833.33±288.67	1000±0	833.33±288.67	1000±0	1333.33±288.67	1500±0
Ph	6.33±0.29	6.5±0	7.5±0.5	7.66±0.29	6.83±0.29	7±0.5	6.5±0	6.5±0.5
DO_2	4.79±0.28	4.51±0.28	6.34±0.36	5.92±0.28	5.35±0.28	5.07±0.28	4.51±0.56	4.47±0.36
DCo_2	6.16±0.35	4.84±0.35	3.81±0.25	2.75±0.41	3.96±0.44	3.55±0.56	7.92±0.35	7.48±0
Carbonate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Bicarbonate	6.66±2.88	13.33±2.88	Nil	Nil	5±0	6.66±2.88	15±5	18.33±2.88
Calcium	18.33±2.88	20±0	8.33±2.88	10±0	13.33±2.88	15±0	21.66±2.88	21.66±5.77
Magnesium	16.66±2.88	16.66±5.773	6.66±2.88	8.33±2.88	3.33±2.88	5±0	13.33±2.88	15±0
Chloride	49.7±7.1	54.43±4.91	28.4±7.1	33.13±4.09	47.33±4.099	49.7±7.1	63.9±7.1	68.63±4.099
Sulphate	79.68±0.783	81.6±1.357	72.96±0.783	76.8±0.96	67.2±1.752	69.84±0.919	60.72±1.639	63.36±1.357
Phosphate	0.61±0.11	0.66±0.12	0.52±0.18	0.56±0.13	0.38±0.11	0.42±0.05	0.30±0.05	0.36±0.20
Nitrate	35.5±0.55	36.22±0.60	30.25±1.25	31.04±1.22	22.20±1.50	23.44±0.22	18.80±1.20	19.80±1.25

Table.4. Chemical parameters of Aryanambi temple pond during the study period Nov.2012-Feb.2013

Parameters	Nov.2012		Dec.2012		Jan.2013		Feb.2013	
	Aryanambi temple pond		Aryanambi temple pond		Aryanambi temple pond		Aryanambi temple pond	
	Surface Water	Bottom water	Surface water	Bottom water	Surface water	Bottom water	Surface water	Bottom water
Total solids	2166.66±288.67	2333.33±288.67	1666.66±288.67	2000±0	1333.33±288.67	1500±0	1833.33±288.67	2166.66±288.67
Ph	6.6±0.29	6.83±0.29	7.83±0.29	8.0±0	7.33±0.29	7.5±0.5	6.83±0.29	7.0±0.5
Do_2	4.08±0.36	3.94±0.28	5.49±0.36	5.07±0.28	4.93±0.36	4.79±0.28	3.80±0.36	3.66±0.28
Dco_2	12.43±0.22	10.78±0.56	6.60±0.44	6.45±0.25	8.80±0.44	7.33±0.25	14.41±0.75	13.64±0.44
Carbonate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Bicarbonate	13.33±2.88	20±0	5±5	6.66±2.88	10±0	11.66±2.88	21.66±2.88	23.33±2.88
Calcium	20±5	23.33±2.88	11.66±2.88	13.33±2.88	15±0	16.66±2.88	25±0	28.33±2.88
Magnesium	20±0	23.33±2.88	10±0	11.66±2.88	8.33±2.88	10±0	16.66±2.88	21.66±5.773
Chloride	56.80±7.1	59.16±8.198	35.5±7.1	40.23±4.099	35.5±7.1	42.6±7.1	73.36±4.099	85.2±7.1
Sulphate	95.76±0.919	98.16±1.44	90.0±0.919	93.36±0.919	85.44±0.783	87.36±0.96	74.4±0.554	78.08±0.554
Phosphate	0.80±0.08	0.84±0.15	0.77±0.08	0.79±0.13	0.62±0.11	0.65±0.12	0.54±0.12	0.58±0.16
Nitrate	36.12±1.02	37.54±0.88	34.0±0.50	34.92±0.18	25.55±0.15	28.22±0.80	20.40±0	22.10±0.22

Fig.1.Showing Temperature variation in Pond A

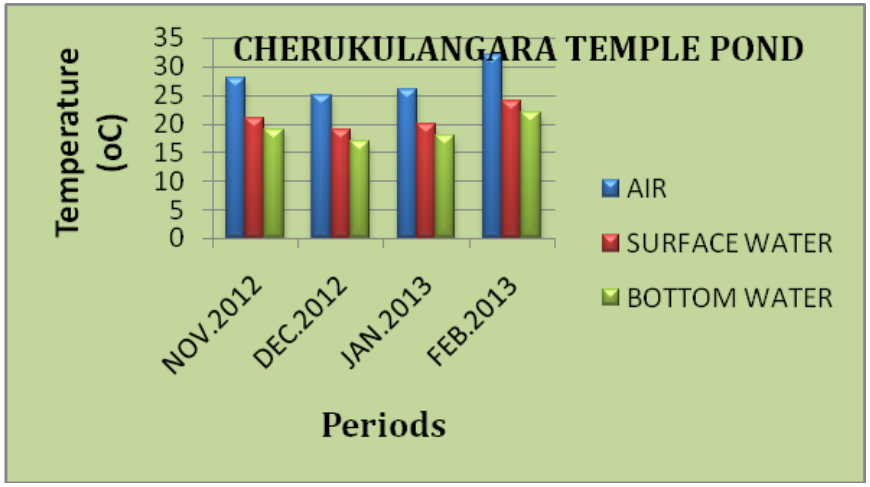


Fig.2.Showing Temperature variation in Pond B

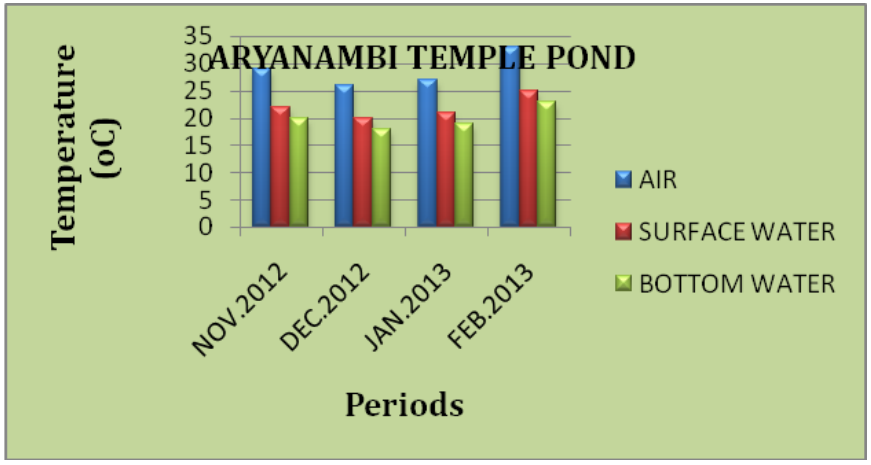


Fig.3.Showing Disssolved oxygen in Pond B

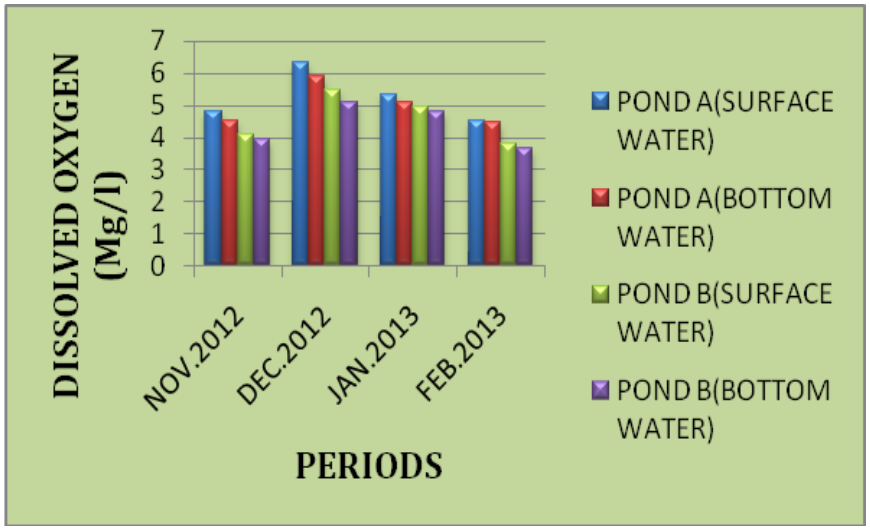


Fig.5. pH in Pond A and B

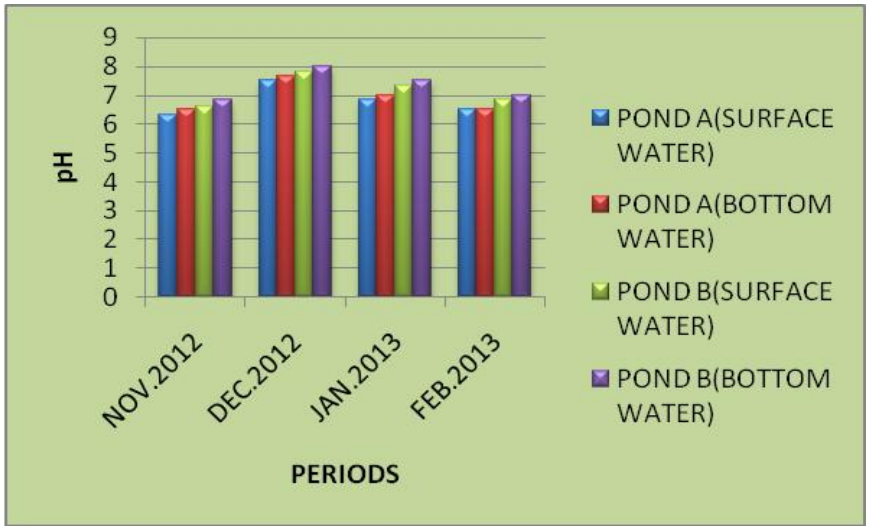


Fig.4.Total solids in Pond A and B

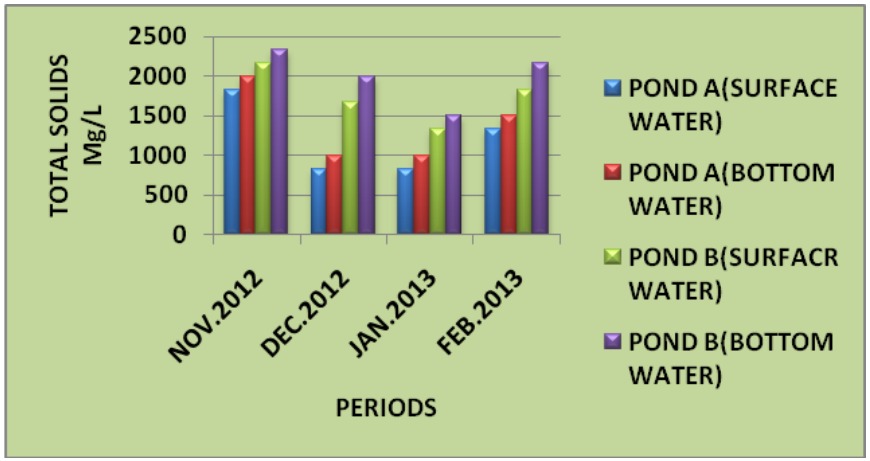


Fig.6.Dissolved carbondioxide in Pond A and B

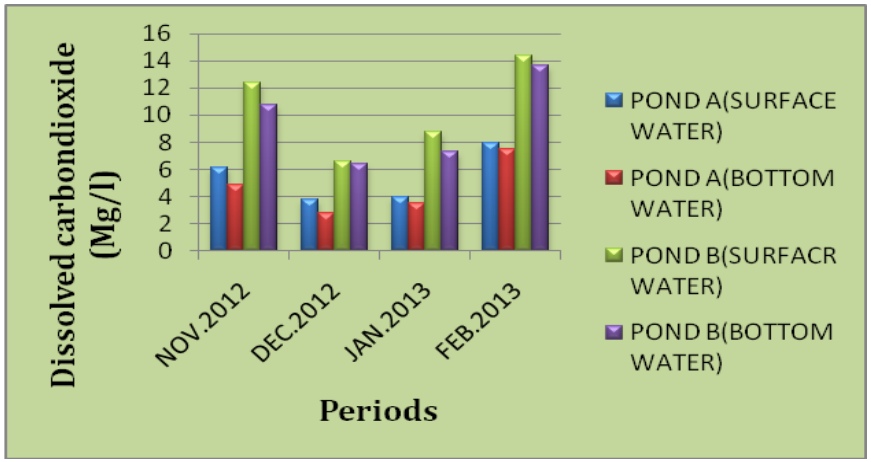


Fig.7.Bicarbonate in Pond A and B

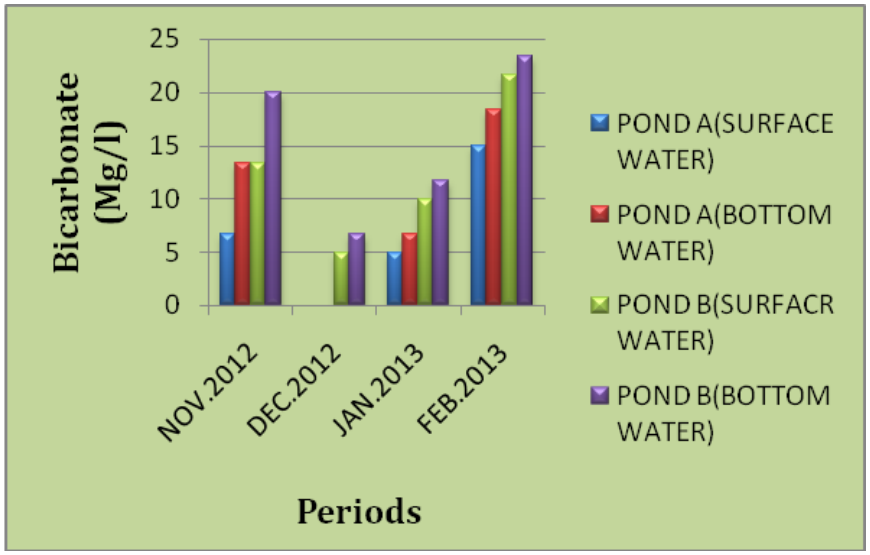


Fig.8.Calcium in Pond A and B

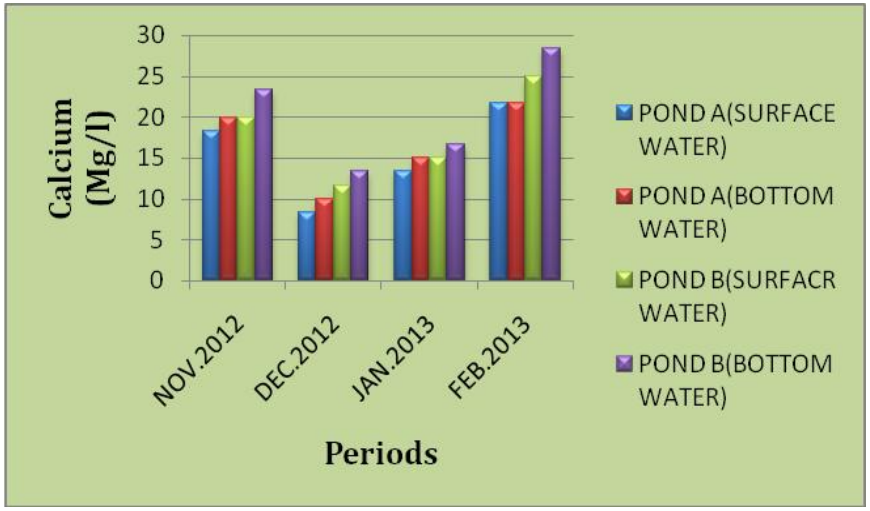


Fig.9.Phosphate in Pond A and B

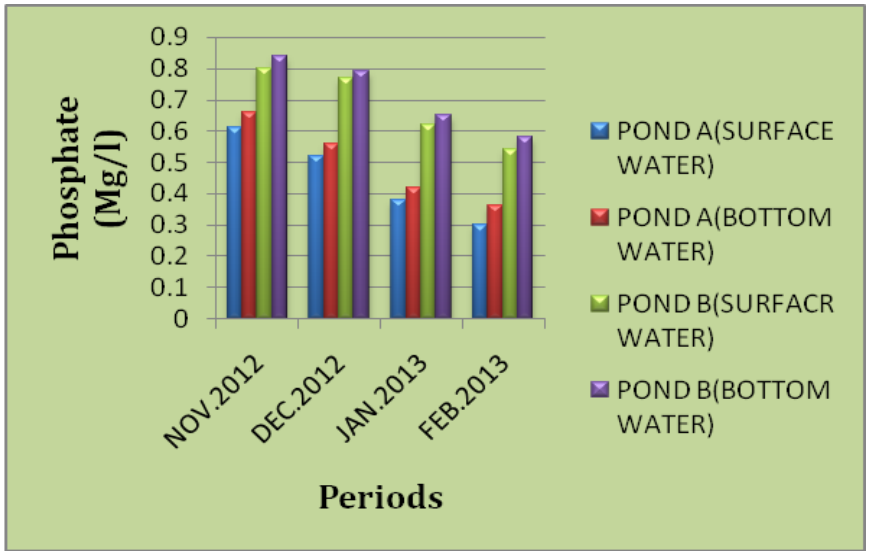


Fig.10.Magnesium in Pond A and B

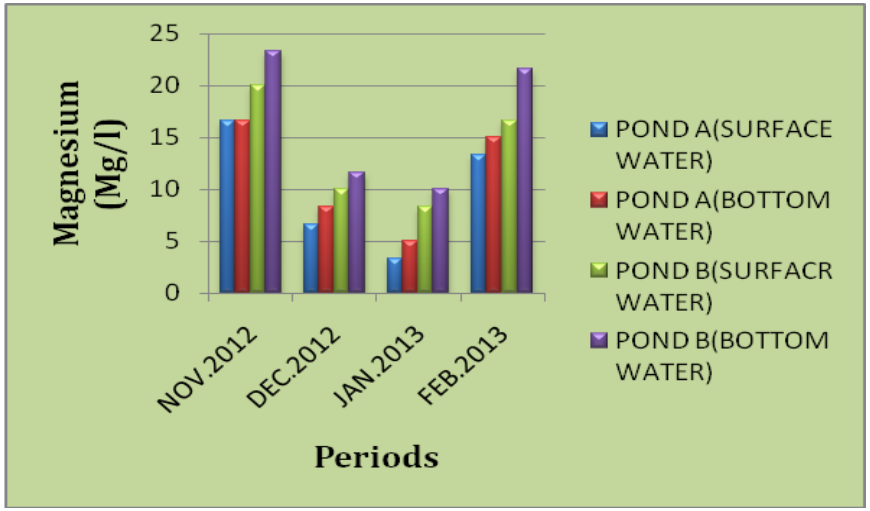


Fig.11.Chloride in Pond A and B

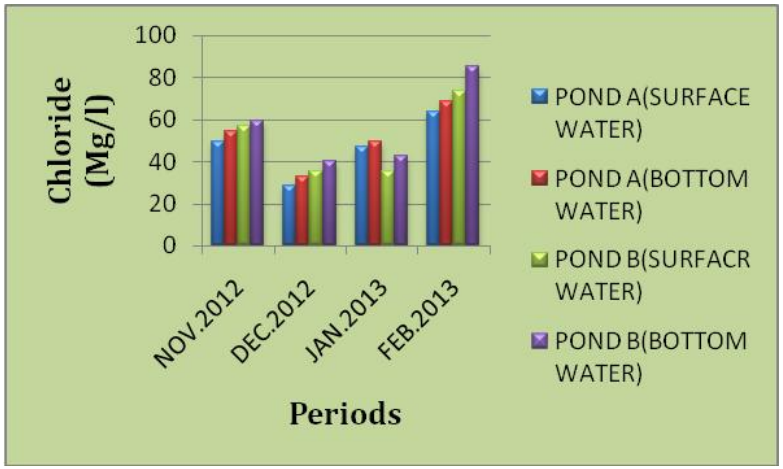


Fig.12.Sulphate in Pond A and B

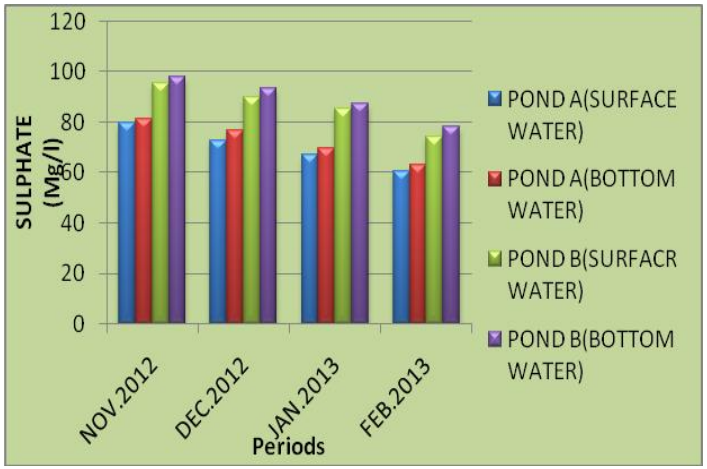
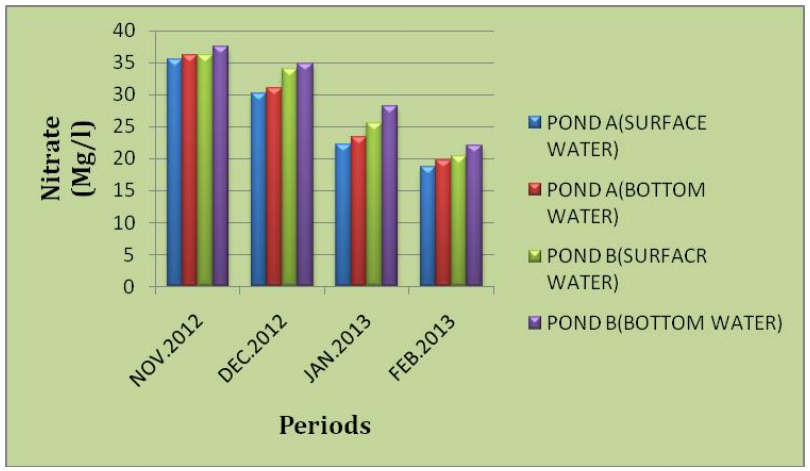


Fig.13.Nitrate in Pond A and B



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SCREENING AND DETERMINATION OF ANTIOXIDANT SCAVENGING ACTIVITY OF *PIPER LONGUM* AND *EUCALYPTUS CAMALDULENSIS*

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ABSTRACT

The herbal medicine is used from time immemorial to treat various common ailments like cold, cough and gastric problems. India has various system of treatment like Ayurveda, Siddha, Unani and Homeopathy which are devoid of side effects and treat the person with higher benefits. *Piper longum* fruits and *Eucalyptus camaldulensis* Dehnh., leaves has been used as spices and for medicinal oil preparation for various treatment. We have made an attempt to elevate the importance of these herbal sources by understanding the antioxidant scavenging activity. We have extracted and processed the plants fruit and leaves using different solvents in 1:10 dilution such as petroleum ether, chloroform ethanol, hexane, methanol and distilled water. It was found that ethanol and methanol extract of *Piper longum* and *Eucalyptus camaldulensis* Dehnh., has higher antioxidant activity when compared with the standard Vitamin C and IC_{50} value was observed to be nearly 70µg/ml for all the tests.

Key words: *Piper longum, Eucalyptus camaldulensis* Dehnh., $DPPH^{\bullet}$, $ABTS^{\bullet+}$, FRAP.

1. INTRODUCTION

Cold and cough is always considered as drastic problem which everyone suffers in all parts of world. India a reservoir for various herbs uses plants and their products to treat the common problems time immemorial. The World Health Organization (WHO) has found that out off 3000 plants most of them are available in India and the usages of these plants are nearly by 80 % of the world population (WHO report, 2009). With an increase rate of over exposure towards various antibiotics has created an environment of multidrug resistance microbes such as *Escherichia coli, Klebsiella pneumoniae, Aeromonas* sp., *Mycobacterium tuberculosis, M. leprae*, *Candida* sp., etc. (Waters and Basseler, 2005). This has made the scientists to explore new drugs either using plant sources like medicinal herbs or their parts or chemical sources. Due to the concern related to the side effects by the usages of chemical drugs (Hassan, 2012) made a shift in the path of drug usages and caused the public to use herbal medicinal sources for the treatment like Ayurveda, Siddha, Unani and Homeopathy.

In the current research we have chosen *Piper longum* fruits and *Eucalyptus camaldulensis* Dehnh., leaves as the source with medicinal property for treating the common problem of cold and cough. *Piper longum* belongs to the *Piperaceae* family and is thought to originate from South East Asia and *Eucalyptus* sp. commonly found in the dry and hilly areas are having many medicinal roles in Ayurveda and in modern medicine. Before evaluating any medicinal plant, it is essential to understand its antioxidant potential because there are various free radicals released by our body and by various products intake (Abrahim *et al.,* 1993; Gupta and Ray, 2004; Kumar *et al.,* 2010; Arawwala *et al.,* 2011). It has also been found that these free radicals remain as an adjuvant in causing cancer like breast cancer. These plants are used commonly as spices or for inhalation treatment for curing the common ailment and it is essential to evaluate the antioxidant potential of these drugs as when used and inhaled could sometime reach the blood stream either by absorption from the tissues or from the stomach (Manoj *et al.,* 2004; Sadlon *et al.,* 2010; Chahal *et al.,* 2011). We have aimed to understand the scavenging potential of these plants as they are commonly used in India as a source for treating common cold and cough problems in various parts of India.

2. MATERIALS AND METHODS

2.1. Collection of herbal plants

Piper longam fruits and *E. camaldulensis* Dehnh., leaves were collected from in and around Coimbatore and subjected for plant authentication at Botanical Survey of India, Coimbatore, Tamil Nadu.

2.2. Processing of Plant fruits and leaves using cold percolation method

The plant leaves and stems were processed using cold percolation method as described by Adonizio *et al.* (2008). The cold percolation method helps to retain the medicinal property of any

medicinal plant. The plant leaves and fruits were stored in dark for a period of 2-3 weeks and were powdered. The powders of plant leaves and fruits were sieved and stored in dark bottle. The powdered plant leaves and fruits were weighed and mixed with different solvents in 1:10 dilution in increasing order of polarity such as petroleum ether, chloroform ethanol, hexane, methanol and distilled water.

They were shaken well during the process to avoid fungal contamination and filtered using muslin cloth. The filtrate was kept in watch glass in dark for evaporation and scrapped powders were stored in dark bottle as they are light sensitive. The extracts were subjected to antioxidant scavenging activity and subjected for future research against pathogens.

2.3. Evaluating the various antioxidant properties of extracted Piper longum and Eucalyptus camaldulensis Dehnh. by in vitro free radical scavenging activity

2.3.1. DPPH$^{\bullet}$ scavenging activity

The 2,2-diphenyl-1picryl hydrazyl (DPPH$^{\bullet}$) scavenging activity of *P. longum* and *E. camaldulensis* Dehnh. is performed using the method described by Blois, in 1995. DPPH$^{\bullet}$ is scavenged by antioxidants through the donation of a proton forming the reduced DPPH$^{\bullet}$. Various concentrations of samples were taken along with Vitamin C as standard in different test tubes. The volume was adjusted to 500 µL by adding methanol and 5 mL of 0.1 mM methanolic solution of DPPH was added to these test tubes and vortexed. The tubes were allowed to stand at room temperature for 20 min. The control was prepared as above without any extract and methanol was used for the baseline correction. The color change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517 nm. The percentage of inhibition radical scavenging activity was measured by the formula:

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

The percentage inhibition vs. concentration was plotted and the concentration required for 50 % inhibition of radicals was expressed as IC_{50} value.

2.3.2. ABTS$^{\bullet+}$ radical scavenging activity

The test was based on the relative activity of antioxidants to quench the radical cation ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)$^{+}$ was done using the method described by Re *et al.* (1999). ABTS$^{\bullet+}$ decolorisation assay involves the generation of the ABTS$^{\bullet+}$ chromophore by the oxidation of ABTS$^{\bullet+}$ with ammonium per sulphate. It is applicable for both hydrophilic and lipophilic compounds. The reaction was initiated by the addition of 1.0 mL of diluted ABTS to 10 µL of different concentration of extract with high antibiofilm activity of the sample or 10 µL of methanol serve as control. The absorbance was read at 734 nm. Percentage inhibition was calculated by the formula

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

2.3.3. Hydrogen Peroxide scavenging activity

The hydrogen peroxide scavenging activity was measured in terms of a decrease in the absorbance as 230 nm in spectrophotometer using the method described by Ruch *et al.* (1989). A solution of H_2O_2 was prepared in phosphate buffer and the H_2O_2 concentration was determined using spectrophotometer at 230 nm wavelength. Various concentrations of plant extracts were added to H_2O_2 and incubated for 10 min. The absorbance at 230 nm was determined against a blank containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 and standard compound Vitamin C was calculated using the formula:

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

2.3.4. Hydroxyl radical scavenging activity

Hydroxyl radicals were generated from ferrous ammonium sulphate and EDTA were determined against the scavenging activity of the plant extracts using the method described by Klein *et al.* (1991). Various concentration of plant extracts were added with 1 mL of iron-EDTA solution (0.13 % ferrous ammonium sulphate and 0.26 % EDTA), 0.5 ml of EDTA solution (0.018 %), and 1 mL of DMSO (0.85 % v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5ml of ascorbic acid (0.22 %) and incubated at 80 to 90 ^{o}C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1 mL of ice-cold tri-chloro acetic acid (TCA) (17.5 % w/v). About 3 mL of Nash reagent (75 g of ammonium sulphate, 3 ml of glacial acetic acid and 2 mL of acetyl acetone were mixed and make up to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. This was detected by their ability to react with ascorbic acid to produce yellow color complex which was measured at 412 nm against reagent

blank. The percentage of hydroxyl radical scavenging activity is calculated by the following formula

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

3.2.5. Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of sample was determined using ferric reducing antioxidant power (FRAP) using the method described by Benzie and Strain, 1996. The stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mm $FeCl_3$, $6H_2O$ and 0.3 M acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 mL TPTZ solution, 2.5 mL ferric chloride solution and 25 mL acetate buffer. It was freshly prepared and warmed to 37 °C. 900 µL FRAP reagent were mixed with 90 µL water and 30 µL test sample/ethanol/distilled water/standard antioxidant solution. The reaction mixture was then incubated at 37 °C for 30 min and the absorbance was recorded at 595nm. An intense blue color complex were formed when ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex were reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded. The calibration curve was plotted with absorbance at 595 nm vs concentration of ferrous sulphate in the range 0.1mM ethanol solutions. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidants.

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

3. RESULTS AND DISCUSSION

The collected *P. longum* fruit and *E. camaldulensis* Dehnh., leaves were taken to Botanical Survey of India, Coimbatore and authentication no. for the plant is BSI/SRC/5/23/2013-14/Tech./2089. The processing and extraction of the plants fruit and leaves were done accordingly and the ethanolic and methanolic extract of *P. longum* and *E. camaldulensis* Dehnh. were subjected for further scavenging activity analysis based on the work done by Kumar and Laxmidhar (2011).

3.1. Evaluating the various antioxidant properties of extracted Piper longum and Eucalyptus camaldulensis Dehnh. by In vitro free radical scavenging activity

The ethanol and methanol extracts of *Piper longum* and *Eucalyptus camaldulensis* Dehnh., were carried out with antioxidant test and identified that the free radical of the extract were found to have high percentage of inhibition against $DPPH^{\bullet}$, $ABTS^{\bullet+}$, hydrogen peroxide, hydroxyl and FRAP. Vitamin C served as the standard for all the antioxidant assays carried out in the study and when compared the ethanol and methanol extracts of *P. longum* and *E. camaldulensis* Dehnh., found to relatively higher and similar to the standard vitamin C. The results were observed to have higher percentage of inhibition for the extracts and the IC_{50} value was observed as 70 µg mL^{-1} (as shown in Fig. 1, 2, 3, 4 and 5) which was found to be similar to the results of Kumar and Laxmidhar (2011).

This shows that *P. longum* and *E. camaldulensis* Dehnh. has higher scavenging activity as compared with the previous results of Kumar and Laxmidhar (2011). Based on the experimental outcome of various researchers, the antioxidant activities, antimicrobial, antitumour and etc., of the fruit of *P. longum* showed higher activities and proved to be useful in many Ayurvedic preparations for treating various ailments (Manoj *et al.,* 2004; Chahal *et al.,* 2011). Antioxidant activities were measured using FRAP, $DPPH^{\bullet}$, superoxide anion, nitric oxide and hyroxyl radical scavenging assays was also found to higher as the result obtained in the current research (Abrahim *et al.,* 1993).

4. CONCLUSION

This proves that both the plants *P. longum* and *E. camaldulensis* Dehnh., have higher scavenging activity using the ethanolic and methanolic extract of plant fruits and leaves. This shows that these plant extracts can be further studied in future to understand the antibacterial activity as well as can serve as a potent drug in future pharmaceutical research for treating various common diseases and in cancer research.

ACKNOWLEDGEMENT

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Fig. 1. DPPH$^{\bullet}$ radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.

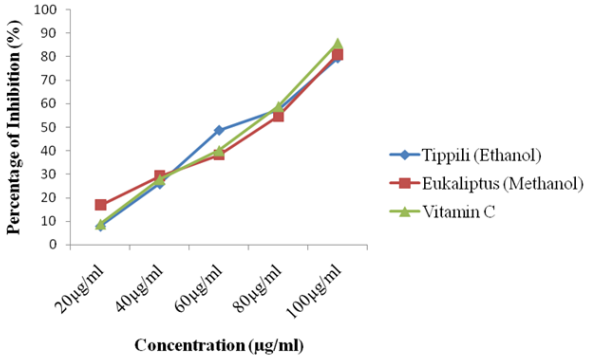


Fig. 2. ABTS$^{\bullet+}$ radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.

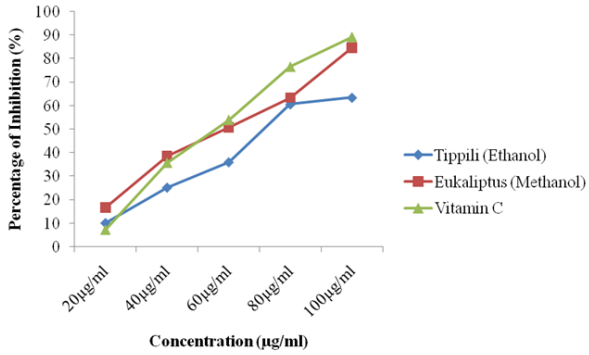


Fig. 3. Hydroxyl radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.

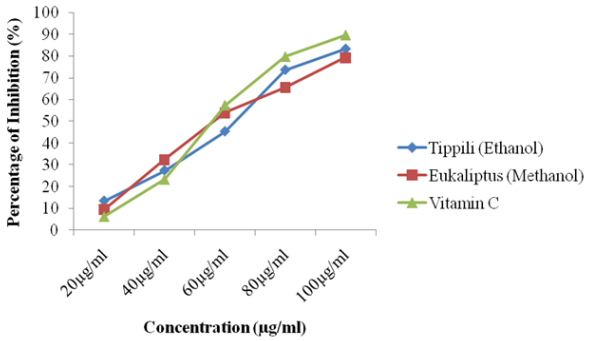


Fig. 4. Hydrogen Peroxide radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.

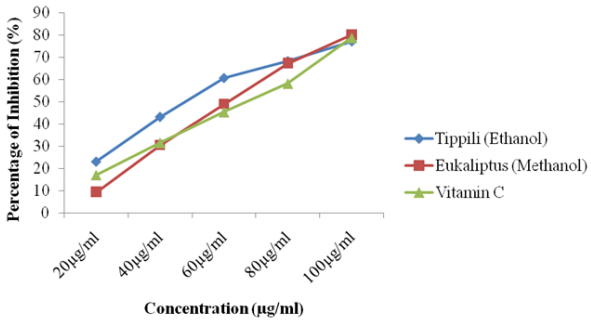
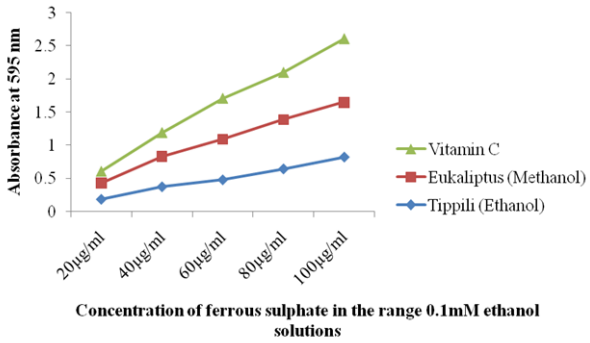


Fig. 5. FRAP radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.



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STUDIES ON TOXICITY OF CERTAIN PLANT EXTRACTS TO LARVAE OF THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*, WITH A NOTE ON THEIR EFFECT ON DEVELOPMENT OF THE MOSQUITO LARVAE

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ABSTRACT

A study was carried out to reveal the toxicity of crude extracts obtained from leaves of herbs to the developmental stages of mosquito, *Aedes aegypti*. Further, experiments were conducted to find out the effect of the plant leaf extracts on certain indices of development of mosquito larvae viz., development period, rate of population, rate of emergence, survival rate and growth index. LC_{50}/ 24 hours values of four plants to I, II, III, IV instars and pupae of Aedes aegypti were also calculated. The data indicated that there was a considerable increase in the LC_{50}/24 values with the age of larvae. Compared to control period of development, form I instar to pupa, extended in the larvae reared in medium of plant extracts. Survival rate and growth index for mosquito larvae were remarkably reduced when treated with plant extracts. To conclude, the leaf extracts of the plant could be used for the control of mosquito larvae. *Kirganelia reticulata* leaf extract found to possess relatively higher toxicity.

Keywords: *Aedes aegypti,* Mosquito larvae, LC_{50}

1. INTRODUCTION

Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to human (Kovendan and Murugan, 2011). The effectiveness of vector control has de- clined because of the reduced effectiveness of insecticides caused by the emergence of resis-tance in mosquitoes against the currently used insecticides (Chandre *et al.*, 1998). The application of easily degradable plant compounds is considered to be one of the safest methods to control insect pests and vectors (Alkofahi *et al.*, 1989).

In recent years there has been much interest in natural insecticides derived from plants which are biodegradable, easily available at low cost, and safe for human health. Various studies on the natural plant products as larvicides against mosquito vectors have been reported [Pizarro *et al*., 1999; Singh *et al*., 2001; Singh *et al*., 2005, 2006, 2007, 2010). However, more concerted efforts would be needed to make these environment friendly compounds viable for field use and for large scale vector control operations.

2. MATERIALS AND METHODS

2.1.Test Animal

The larvae of mosquitoes were collected from nearby water bodies and reared in the laboratory untill they emerge into adults. They were then identified in to their species and maintained in separate cages.The adult mosquitoes were maintained in a cage of size 1 cu.ft.A total number of 20 mosquitoes with a sex ratio of 1:1 were regularly maintained in the cage for continued supply of eggs.The adult female mosquitoes were fed with blood of chick in every alternative day. Both male and female were provided with 10%glucose solution. The cotton was always kept moist with the solution and changed everyday.An egg trap (cup) lined with filter paper containing filtered water was always placed at a corner of the cage. This arrangement made collection of eggs easier.The larvae were reared in plastic cups.They were daily provided with commercial fish food (Lyimo et al.,1992) *ad libitum.*Water was changed alternate days.The normal cultures as well as those kept for experimental purpose were covered with muslin cloth which will prevent contamination through foreign mosquitoes.

2.2. Test compounds

Crude water leaf extracts of herbal plants, *Kirganelia reticulata*, *Pavetta indica, Cleome viscosa*, Vedathi (Plate I-IV) were used as toxicants in the present study.The leaves were picked out, Cleaned and air dry under the shade till they become fit to be powdered.A non quantity of finally powdered leaves of any type of plant was taken in a container with 200 ml of filtered tap water (Unchlorinated) and stirred for 1 hour with

magnetic stirrer and kept for 24 hours. The mixture was then filtered through whatman no:1 filterpaper. The filterate was used as the experimental medium.The amount of the leaf powder taken at a time was in proportion to the concentration of the medium required.For example,1% medium of any of the sample was prepared by dissolving 2 gm of leaf powder in 20ml of filtered tap water.

2.3.Bioassay Test

To test the efficacy of crude water extracts of the on *Aedes aegypti* at different developmental stages *viz.,* I, II, III and IV instar and pupa were subjected to bioassay experiments. Different concentrations (0.2 - 5%) of any of the test compounds were prepared using filtered tap water as described earlier. Clean plastic cups were used as test containers. 20 larvae at a particular stage of development were exposed to 200ml of a particular concentration of test solution (plate 7-10). Mortality rates of larvae were recorded after 24 hours. Five or more concentrations of attest compound giving between 0 and 100% mortality for larvae at different instar stages were recorded. Parallel control was maintained. Three replicate were done at each concentration. In recording the percentage mortality for each concentration, the moribund and dead larvae were combined. For computing LC_{50}/24hrs the data were subjected to Finney's method of probit analysis (Finney, 1971).

2.4.Toxicity of the plant leaf extracts to the larvae of Aedes aegypti

The larvae of *Aedes aegypti* at different instar stages I, II, III, IV and pupae were exposed to medium contain serious of concentrations of extracts obtained from leaves of the test toxicant plants and doses giving mortality of larvae ranging from 0 to 100% was recorded. The data were objected to finney's method of probit analysis to derived median lethal concentration which was expressed in terms of LC_{50}/ 24 hours.

3. RESULTS AND DISCUSSION

LC_{50}/24 hours values of *Pavetta indica* to I, II, III and IV instar larvae and pupae of *Aedes aegypti* were 1.6099, 1.704, 1.8092, 1.8776 and 2.3371%, respectively.*Kirganelia reticulate* showed higher toxicity compared to that any other plants used in the present study. This was exhibited from the low LC_{50}/24 hr values recorded: 0.6684, 0.6968, 0.7061, 0.7462, 0.893% for I, II, III and IV instar larvae and pupae,respectively.Median lethal concentration of *Cleome viscosa* to the mosquito larvae were 1.5764, 1.828, 2.0231, 2.2099 and 2.4391 %, respectively. And those of *Vedathi* were 1.6095, 1.7825, 1.9237, 2.3755 and 2.532% for I, II, III and IV instar larvae and pupae respectively (Table.1and Fig.1).

A notable observation of the present study was that susceptibility of different larval stages to the leaf extracts of the plants varied according to their age. Susceptibility of first instar larvae to the test compound was always higher compared to that of the larvae at other stages of development which were in the order of II III IV Pupae in their response as the LC_{50}/24 hours values to the mosquito larvae were in the order of I II III IV Pupae.

3.1.Effect of plant leaf extracts on pupation and emergence of Aedes aegypti

Medium contain Sublethal dose of leaf extracts of any of the plants was individually introduced with 30 I instar larvae of the mosquito. They were provided with fish food *ad libitum* till emergence. The medium was checked every 24 hours to record number of larvae moulted to the next stage and dead if any, were removed.From the data obtained percentage of pupation and adult emergence was calculated. The experiment was continued till the last larvae emerged into adult. The experiment was repeated thrice of every plant extract and a parallel control was maintained.

Mean percentage of larvae pupated in Sublethal medium of *Pavetta indica, Vedathi* and *Cleome viscosa* was 85, 86.66 and 92.2 were those reared in control showed 95.53% among the plant extracts used, *Kirganelia reticulata* showed highest effect of pupation by allowing only 80% of larvae to pupate and 43.33% emerged into adult. The percentage of emergence was also considerably low among the larvae treated with extracts of *Pavetta indica, Vedathi* and *Cleome viscosa* as 46.66%, 46.83% and 53.33% respectively (Table 2 and Fig 2).

3.2.Effect of the plant extracts on indices of development of Aedes aegypti

The mosquito larvae were reared in the Sublethal medium of extracts obtained from plants *Pavetta indica, Kirganelia reticulata, Cleome viscosa,* and *Vedathi*. Daily record of their transmission from one stage to another was maintained till the larvae emerged into adult.

The larvae reared in control medium took 6.5 days for pupation, whereas those grown in leaf extract of *Kirganelia reticulata* pupated on day 10.Extracts of *Pavetta indica Vedathi* and *Cleome viscosa* also considerably prolonged the development period to 7.65, 7.36 and 7.38 respectively compared to that of the control. This value was obtained by dividing percent survival of larvae at pupation by development period. Growth Index for the mosquito larvae at control medium was 15.40. This was considerably less for those *Aedes aegypti* larvae grown in medium made of leaf extracts of *Kirganelia reticulata, Pavetta indica, Cleome viscosa* and *Vedathi*, 10, 11.11, 11.77 and 12.49, respectively(Table 3 and Fig.3).

Larvicidal property of *Kirganelia reticulata, Pavetta indica, Cleome viscosa* and *Vedathi* to different instar stages and pupae *Aedes aegypti,* was evidently proved from the median lethal concentration obtained in the present study. LC50/24 hour values of *Kirganelia reticulata* to I, II, III, IV instar stages and pupae were 0.6684, 0.6968, 0.7061, 0.7461 and 0.893%, respectively and that of *Pavetta indica* 1.6099, 1.704, 1.746, 1.8776 and 2.3371%; *Cleome viscosa* 1.5764, 1.8328, 2.0231, 2.2099 and 2.4391% and *Vedathi* 1.6095, 1.7825, 1.9237, 2.3755 and 2.532%, respectively. Though direct report on the toxicity of the plant selected for this study are not available, the result could be compared to those of investigations conducted using products obtained from other plant species.

4. CONCLUSION

In current study, a considerable increase in the LC_{50} values with the age of larvae was observed i.e early instar larvae were more susceptible to the plant extracts than late instars. It was found that pupae and fourth instar larvae, in particular,were more tolerant to the toxicants than early instars.The decrease in susceptibility may be attributed to increased size and weight of the older larvae(Gbolade, 2001).

Aedes aegypti larvae reared in sublethal concentration of the herbs *Kirganelia reticulata, Pavetta indica, Cleome viscosa, Vedathi* used in the present study showed prolonged pre-adult development time in comparison to control. Larvae in control medium pupated in 6.5 days, whereas those exposed to extracts of the plants took 10, 9, 8.5 and 8 days, among the treated compared to that of control (95.53%).The lowest percentage of pupation (80%) was found at medium contained leaf extracts of *Kirganelia reticulata.*Computation of Growth Index also confirmed the delayed effect of plant leaf extracts on the development of the mosquito larvae.

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Table. 1. LC_{50}/24 hour (%) value of Plants (1,2,3 and 4) on the developmental stages (I - IV instars and Pupae) of *Aedes aegypti.*

S.No	Plants	I Instar larva	II Instar larva	III Instar larva	IV Instar larva	Pupa
1	*Pavetta indica*	1.61 (1.33-1.9299)	1.70 (1.40-2.07)	1.81 (1.53-2.14)	1.88 (1.59-2.24)	2.33 (2.08-2.60)
2	*Kirganilea reticulata*	0.67 (0.56-0.81)	0.70 (0.58-0.86)	0.71 (0.60-0.81)	0.75 (0.60-0.92)	0.90 (0.80-1.004)
3	*Cleome viscosa*	1.58 (1.29-1.90)	1.83 (1.53-2.17)	2.02 (1.70-2.40)	2.21 (1.93-2.50)	2.44 (2.12-2.80)
4	*Vedathi*	1.61 (1.32-1.94)	1.78 (1.45-2.23)	1.92 (1.61-2.28)	2.21 (2.07-2.71)	2.53 (2.21-2.91)

Fig.1. LC_{50}/24 hour (%) value of Plants (1,2,3 and 4) on the developmental stages (I - IV instars and Pupae) of *Aedes aegypti.*

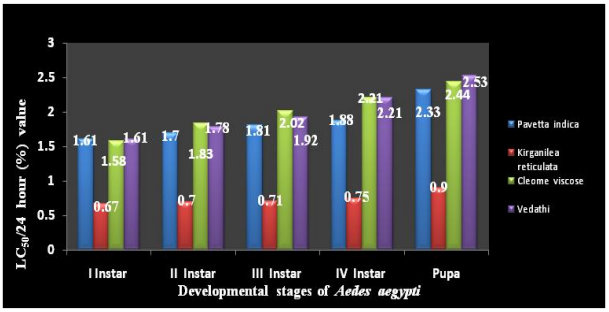


Fig.2. Change in per cent pupation/emergence of *Aedes aegypti* larvae reared in medium contained Sublethal concentration of plant leaf extracts.

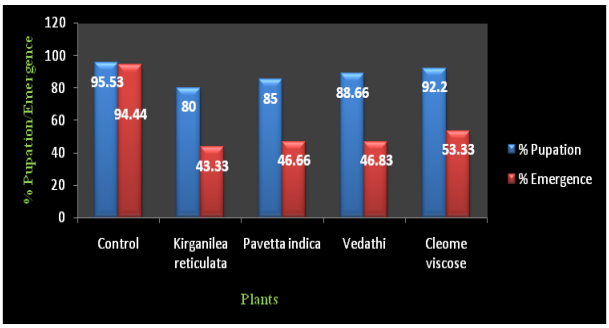
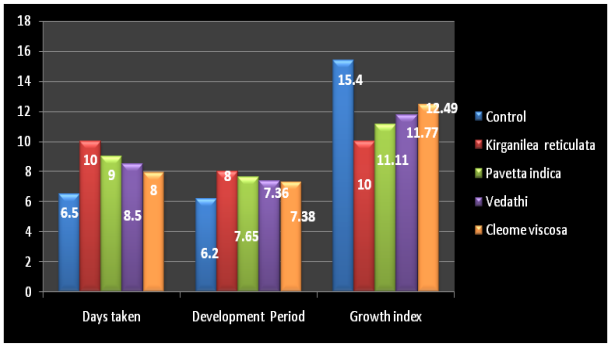


Table.3. Change in the development period of *Aedes aegypti* larvae reared in Sublethal medium of plant extracts and control.

Plants	Days taken	Developmental Period	Growth index
Control	6.5	6.20	15.40
Kirganilea reticulata	10.0	8.0	10
Pavetta indica	9.0	7.65	11.11
Vedathi	8.5	7.36	11.77
Cleome viscosa	8.0	7.38	12.49

Fig.3.Change in the development period of *Aedes aegypti* larvae reared in Sublethal medium of plant extracts and control.



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WITHANIA SOMNIFERA: DETERMINATION OF ANTIOXIDANT SCAVENGING POTENTIAL ACTIVITY

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ABSTRACT

Due to an increase in modernization, people suffer from various stresses related and sexual problems. In India, with various herbal reservoirs, *Withania somnifera* (Ashwagandha) plays an important role in treating the vital and vigour problem as well as in treating sexual problems. We made an attempt to elevate the importance of this herbal source by understanding its antioxidant scavenging activity. We have utilized the powder of this plant fruit and processed using different solvents in 1:10 dilution by dissolving with 3g of powder with 30ml of each solvent such as petroleum ether, chloroform ethanol, hexane, methanol and distilled water. It was found that methanol and chloroform extracts of *W. somnifera*, has higher antioxidant activity when compared with the standard Vitamin C was observed using the graph plotted as the percentage of inhibition of scavenging activity vs concentration and IC_{50} value was observed to be nearly 50 µg/mL for all the tests.

Key words*: W. somnifera*, DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP

1. INTRODUCTION

Vitality and Vigour are the most preferred things in any individual and these things are essential in this modern world to harness various new work. Indian medicine has always believed to provide a source of vitality and vigour with the help of herbal medicinal approach using Ayurveda, Siddha, Unani and Homeopathy as a common way for treating using various herbal preparations of whole plant and their products to treat the common problems time immemorial (Samhita, 1997).

The World Health Organization (WHO) has found that out off 3000 plants most of them are available in India and the usages of these plants are nearly by 80% of the world population (WHO report, 2009). With an increase rate of over exposure towards various antibiotics has created an environment of multidrug resistance microbes such as *Escherichia coli, Klebsiella pneumoniae, Aeromonas* sp., *Mycobacterium tuberculosis, M. leprae*, *Candida* sp., etc. (Waters and Basseler, 2005). This has made the scientists to explore new drugs either using plant sources like medicinal herbs or their parts or chemical sources. Due to the concern related to the side effects by the usages of chemical drugs (Hassan, 2012) made a shift in the path of drug usages and caused the public to use herbal medicinal sources for the treatment.

In the current research we have chosen *Withania somnifera* (Ashwagandha) fruit powder as the source with medicinal property for treating the common problem of vigour and vitality ailments in sexual problems. Among the plants known for medicinal value, the plants of genus *Withania* belonging to family *Solanaceae* are very important for their therapeutic potentials. *W. coagulens, W. simonii, W. adunensis,* and *W. riebeckii* are examples of known in the world and are also known to have medicinal properties (Atal and Kapoor, 1989; Sanyal, 1989; Chopra *et al.,* 1993). *W. somnifera* is one of the major herbal components of geriatric tonics mentioned in Indian systems of medicine. In the traditional system of medicine Ayurveda, this plant is claimed to have potent aphrodisiac, rejuvenative and life prolonging properties. Recent research suggests a possible mechanism behind the increased cytotoxic effect of macrophages exposed to *W. somnifera* extracts (Davis and Kuttan, 2000). Before evaluating any medicinal plant, it is essential to understand its antioxidant potential because there are various free radicals released by our body and by various products intake (Abrahim *et al.,* 1993; Gupta and Ray, 2004; Arawwala *et al.,* 2010; Kumar *et al.,* 2010). It has also been found that these free radicals remain as an adjuvant in causing cancer like skin cancer. This plant are used commonly as spices or as supplement in dietary items like tea and it is essential to evaluate the antioxidant potential of these drugs as observed to have high activity to scavenge the free radicals (Prakash *et al.,* 2002). We have aimed to understand the scavenging potential of this plant as they are commonly used in India as a source for providing energetic booster and to treat sexual vigour and vitality problems in various parts of India.

2. MATERIALS AND METHODS

2.1. Collection of herbal plants

Withania somnifera fruit powder was collected from Aravind Herbal Pvt. Ltd., Rajapalyam, Tamilnadu, India and was subjected for processing in PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore.

2.2. Processing of W. somnifera fruit powder using cold percolation method

The *W. somnifera* fruit powder was processed using cold percolation method as described by Adonizio *et al.* (2008). To retain the medicinal property of any medicinal plant, the cold percolation method is used and in this method, the powder was weighed and mixed with different solvents in 1:10 dilution, *i.e.,* 3 g of powder with 30ml of solvents in increasing order of polarity such as petroleum ether, chloroform ethanol, hexane, methanol and distilled water. They were shaken well at 120 rpm during the process to avoid fungal contamination and filtered using muslin cloth. The filtrate was kept in watch glass in dark for evaporation and scrapped powders were stored in dark bottle as they are light sensitive. The extracts were subjected to antioxidant scavenging activity and subjected for future research against pathogens.

2.3. Evaluating the various antioxidant properties of extracted W. somnifera

2.3.1. DPPH$^{\bullet}$ scavenging activity

The 2,2-diphenyl-1picryl hydrazyl (DPPH) scavenging activity of *W. somnifera* is performed using the method described by Blois, in 1995. DPPH is scavenged by antioxidants through the donation of a proton forming the reduced DPPH$^{\bullet}$. In different test tubes, various concentrations of samples were taken along with Vitamin C as standard. The volume was adjusted to 500 µL by adding methanol and 5 mL of 0.1 mM methanolic solution of DPPH was added to these test tubes and vortexed. The tubes were allowed to stand at room temperature for 20 min. The control was prepared as above without any extract and methanol was used for the baseline correction. The color change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517 nm. The percentage of inhibition radical scavenging activity was measured by the formula.

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

The percentage inhibition vs. concentration was plotted and the concentration required for 50 % inhibition of radicals was expressed as IC_{50} value.

2.3.2. ABTS$^{\bullet+}$ radical scavenging activity

The test was based on the relative activity of antioxidants to quench the radical cation ABTS$^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)$^{+}$ was done using the method described by Re *et al.* (1999). ABTS$^{\bullet+}$ decolorisation assay involves the generation of the ABTS^{+} chromophore by the oxidation of ABTS with ammonium per sulphate. It is applicable for both hydrophilic and lipophilic compounds. The reaction was initiated by the addition of 1.0 mL of diluted ABTS to 10 µL of different concentration of extract with high antibiofilm activity of the sample or 10 µL of methanol serve as control. The absorbance was read at 734 nm. Percentage inhibition was calculated by the formula

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

2.3.3. Hydrogen Peroxide scavenging activity

The hydrogen peroxide scavenging activity was measured in terms of a decrease in the absorbance as 230 nm in spectrophotometer using the method described by Ruch *et al.* (1989). A solution of H_2O_2 was prepared in phosphate buffer and the H_2O_2 concentration was determined using spectrophotometer at 230 nm wavelength. Various concentrations of plant extracts were added to H_2O_2 and incubated for 10 minutes. The absorbance at 230 nm was determined against a blank containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 and standard compound Vitamin C was calculated using the formula:

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

2.3.4. Hydroxyl radical scavenging activity

Hydroxyl radicals were generated from ferrous ammonium sulphate and EDTA were determined against the scavenging activity of the plant extracts using the method described by Klein *et al.* (1991). Different concentrations of plant extract were added with 1 mL of iron-EDTA solution (0.13 % ferrous ammonium sulphate and 0.26 % EDTA), 0.5 ml of EDTA solution (0.018 %), and 1 mL of DMSO (0.85 % v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5ml of ascorbic acid (0.22 %) and incubated at 80 to 90 ^{o}C for 15 min in a water bath. After incubation the reaction was

terminated by the addition of 1 ml of ice-cold tri-chloro acetic acid (TCA) (17.5 % w/v). About 3 mL of Nash reagent (75 g of ammonium sulphate, 3 mL of glacial acetic acid and 2 mL of acetyl acetone were mixed and make up to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. This was detected by their ability to react with ascorbic acid to produce yellow color complex which was measured at 412 nm against reagent with blank. The percentage of hydroxyl radical scavenging activity is calculated by the following formula.

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

2.3.5. Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of sample was determined using ferric reducing antioxidant power (FRAP) using the method described by Benzie and Strain, 1996. The stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mm $FeCl_3$, $6H_2O$ and 0.3 M acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 mL TPTZ solution, 2.5 mL ferric chloride solution and 25 mL acetate buffer. It was freshly prepared and warmed to 37 °C. 900 µL FRAP reagent were mixed with 90 µL water and 30 µL test sample/ethanol/distilled water/standard antioxidant solution. The reaction mixture was then incubated at 37 °C for 30 min and the absorbance was recorded at 595nm. An intense blue color complex were formed when ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex were reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded. The calibration curve was plotted with absorbance at 595 nm vs concentration of $FeSO_4$ in the range 0.1 mM ethanol solutions. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidants.

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

3. RESULTS AND DISCUSSION

The processing and extraction of the *W. somnifera* powder was done accordingly and the methanolic and chloroform extract of *W. somnifera* were subjected for further scavenging activity analysis based on the work done by Prakash *et al.* (2002).

The methanol and chloroform extracts of *W. somnifera* were carried out with antioxidant test and identified that the free radical of the extract were found to have high percentage of inhibition against $DPPH^{\bullet}$, $ABTS^{\bullet+}$, hydrogen peroxide, hydroxyl and FRAP. Vitamin C served as the standard for all the antioxidant assays carried out in the study and when compared the methanolic extracts was higher than chloroform extract of *W. somnifera.* The results were observed to have higher percentage of inhibition for the extracts and the IC_{50} value was observed as 50 µg /mL in all the tests (as shown in Fig. 1, 2, 3, 4 and 5) which was found to be similar to the results of Prakash *et al.*, (2002).

Based on the experimental outcome of various researchers, additionally, levels of reduced glutathione, superoxide dismutase, catalase, and glutathione peroxidase in the exposed tissue returned to near normal values following administration of the extract. The chemo-preventive activity is thought to be due in part to the antioxidant/ free radical scavenging activity of the extract (Prakash *et al.,* 2002). Antioxidant activities were measured using FRAP, DPPH, superoxide anion, nitric oxide and hyroxyl radical scavenging assays was also found to higher as the result obtained in the current research (Abrahim *et al.,* 1993).

An *in vitro* study showed withanolides from *W. somnifera* inhibited growth in human breast, central nervous system, lung, and colon cancer cell lines comparable to doxorubicin. Withaferin A more effectively inhibited growth of breast and colon cancer cell lines than did doxorubicin. These results suggest *W. somnifera* extracts may prevent or inhibit tumor growth in cancer patients, and suggest a potential for development of new chemotherapeutic agents as well as found to inhibit various pathogens leading to act as antimicrobial agents.

4. CONCLUSION

This proves that both the methanolic and chloroform extracts of *W. somnifera* has higher scavenging activity and shows that these plant extracts can be further studied in future to understand the antibacterial activity as well as can serve as a potent drug in future pharmaceutical research for treating various common diseases and in cancer research.

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Fig. 1. DPPH• radical scavenging activity of *W. somnifera*

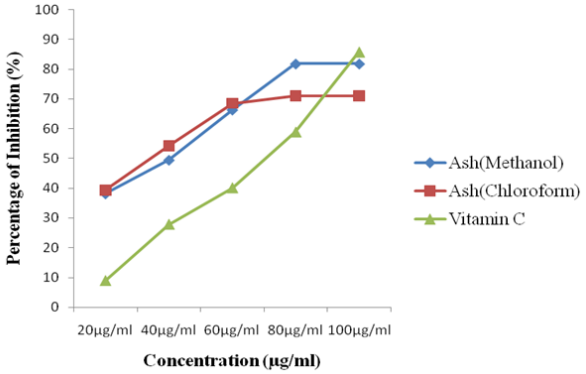


Fig. 2. ABTS•+ radical scavenging activity of *W. somnifera*

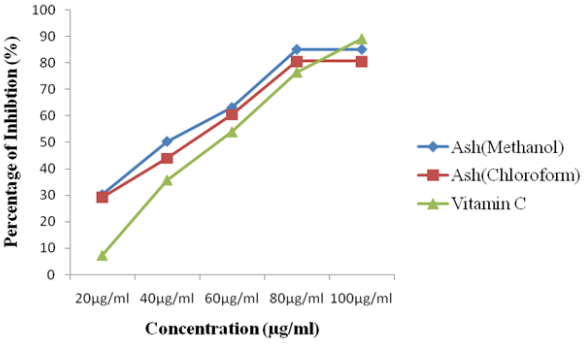


Fig. 3. Hydroxyl radical scavenging activity of *W. somnifera*

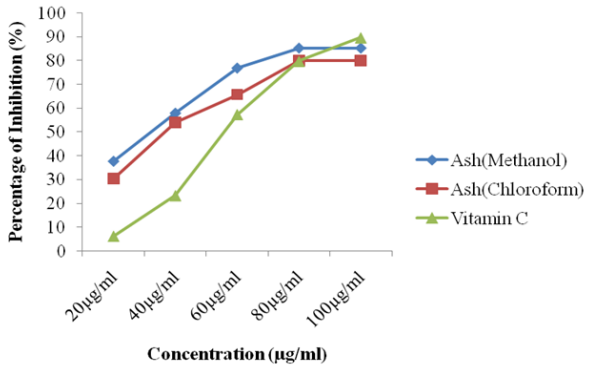


Fig. 4. Hydrogen peroxide radical scavenging activity of *W. somnifera*

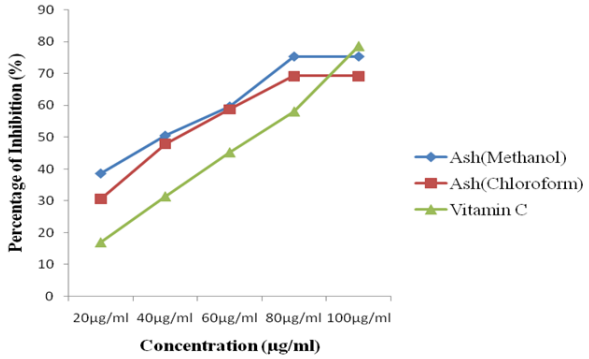
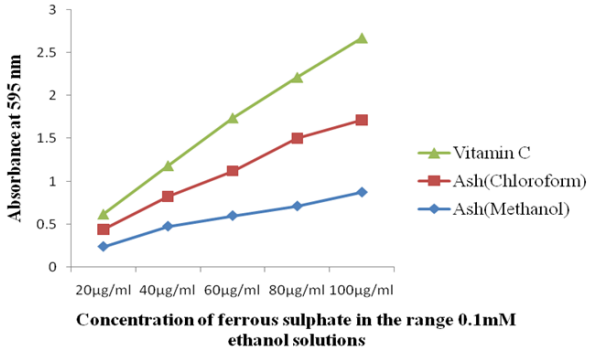


Fig. 5. FRAP radical scavenging activity of *W. somnifera*



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SCREENING OF PHYTOCHEMICALS AND QUANTITATIVE ESTIMATION OF TOTAL FLAVONOIDS AND PHENOLIC COMPOUNDS OF *LAGASCEA MOLLIS* CAV. (ASTERACEAE)

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ABSTRACT

Aim of the study was to analyze the phytochemical constituents and estimation of total flavonoids and phenolic compounds of leaves and roots of the medicinal plant *Lagascea mollis.* Methanol extract of dried leaves and roots of *L. mollis* was prepared by using soxhlet apparatus. The extract prepared was tested for preliminary qualitative phytochemical screening, followed by the quantitative estimation of total flavonoids and phenols by spectroscopy. The selected plant parts were found to contain alkaloids, flavonoids and terpenoids in both parts and saponin is present only in leaf. In quantitative estimation the reports revealed the presence 0.017 and 0.013 mgRE/g extract and 458.91 and 704.50 mgGAE/g extract total flavonoids and phenolic compounds in leaf and root extract respectively. These major phytoconstituents present in this species may be accounted as factors for the medicinal importance of *L. mollis*.

Keywords: *Lagascea mollis,* Asteraceae, flavonoids, phenols.

1. INTRODUCTION

Medicinal plants that have at least one of their parts are being (leaves, stems, barks or roots) used for therapeutic purposes (Bruneton, 1993). World plant biodiversity is the largest source of herbal medicine and still about 60-80% of world population rely on plant based medicines which are being used since the ancient ages as traditional health care system. India is endowed with rich wealth of medicinal plants, which ranked our country in the list of top producers of herbal medicine. In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation (Aqil *et al*., 2006). It is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. Phytochemicals are responsible for medicinal activity of plants (Savithramma *et al.,* 2011), these are non-nutritive chemicals that have protected human from various diseases. Phytochemicals are basically divided into two groups that are primary secondary metabolites based on the function in plant metabolism. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs (Savithramma *et al.,* 2011). The main purpose of the present study was to evaluate the presence of various phytochemicals and quantitative estimation of flavonoids and phenols in leaf and root extract of *Lagascea mollis.*

Lagascea mollis belongs to the family, Asteraceae, commonly known as Seruppadithazhai, Kenathuppoondu in Tamil is native to tropical America. The leaf paste of this plant is given to cuts and wounds. Flowers are given for ear complaints. It helps to control noxious weeds, serve as animal fodder and safe to grow with corn and beans (Alarcon *et al.,* 2007; Koche *et al.,* 2008). It is reported to have antibacterial activity and isolation of flavonol, glyacoside, patulitrin and isopatulitrin as well as dehydrofalcarinone (Chourasia and Rao, 1987; ElNagar and Doskotch, 1979; Bohlmann *et al.,* 1962), ensures the antimicrobial property.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The leaf and roots of *Lagascea mollis* plant were collected from the vicinity of Coimbatore, Tamil Nadu.

2.2. Preparation of extract

The samples were dried at room temperature and further ground in a mortar. About 20 of each part of plant powder was extracted in 100 mL of methanol by soxhlet apparatus. The solvent was concentrated at temperature below 40°C and the resulting extracts were used for determination of phytochemicals.

2.3. Phytochemical screening

The extracts were subjected to preliminary phytochemical tests to determine the groups of secondary metabolites present in the plant material as follows:

2.3.1. Detection of alkaloids (Ciulci, 1994)

To 1 mL of each extract, 2-3 drops of Dragendorff's and Meyer's reagents were separately added. An orange red precipitate/turbidity with Dragendorff's reagent or white precipitate with Meyer's reagent would indicate the presence of alkaloids.

2.3.2. Detection of flavonoids (Sofowora, 1993)

To the 4 ml of each part of the plants extract, a piece of magnesium ribbon was added followed by concentrated HCl drop wise. A colour ranging from crimson to magenta indicated the presence of flavonoids.

2.3.3. Detection of glycosides (Gokhale *et al.,* 2008)

Keller Kiliani test: To the 2 mL of extracts, 1mL of glacial acetic acid with ferric chloride and concentrated sulphuric acid were added. The appearance of blue colour indicates the presence of glycosides.

2.3.4. Detection of saponins (Brain and Turner, 1975)

One ml of extract was taken in a test tube and 5 mL of distilled water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes indicated the presence of saponins.

2.3.5. Detection of tannins (Mace and Gorbach, 1963; Ciulci, 1994)

Two ml of the extracts were diluted with distilled water in separate test tubes and 2-3 drops of 5% ferric chloride ($FeCl_3$) solution was added. A green-black or blue-black colouration indicates the presence of tannins.

2.3.6. Detection of steroids (Ciulci, 1994)

Two mL of the extracts were taken in separate test tubes and evaporated to dryness. The residues were dissolved in acetic anhydride followed by the addition of chloroform. Concentrated sulphuric acid was added by the side of the test tube. Formation of brown ring at the interphase of the two liquids and the appearance of violet colour in the supernatant layer indicated the presence of steroids.

2.3.7. Detection of terpenoids (Salkowski and Balish, 1991)

Five mL of extracts were mixed with 2 mL of chloroform and concentrated H_2SO_4 to form a layer. A reddish brown coloration of the interface shows the presence of terpenoids.

2.3.8. Detection of phenols (Krishnamoorthy, 1988)

Five ml of the concentrated extracts were taken and 2 mL of neutral ferric chloride solution was added. Appearance of violet colour indicates the presence of phenols.

2.4. Quantitative phytochemical analysis

2.4.1. Determination of total flavonoids

The total flavonoid content of samples was determined by following the modified colorimetric method of Zhishen *et al.* (1999). 0.5 ml extract was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% $NaNO_2$ solution. After 6 min, 0.15 mL of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, then 2 mL of 4% NaOH solution was added to the mixture. Immediately distilled water was added to bring the final volume to 5 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was recorded at 510 nm versus prepared water blank. Rutin was used as a standard compound for the quantification of total flavonoid. All the values were expressed as milligram of rutin equivalent (RE) per gram of extract.

2.4.2 Determination of total phenolics

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003). Aliquots of each extract were taken in test tubes and made up to the volume of 1 mL with distilled water. Then 0.5 mL of folin-ciocalteu phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as gallic acid equivalents (GAE).

3. RESULTS AND DISCUSSION

The extractive values, colour and the per cent yield of the leaves and roots of *Lagascea mollis* are given in Table 1. The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical characters of *L. mollis* were investigated and

presented in Table 2. The results showed the presence of alkaloids, flavonoids and terpenoids in both parts and saponin in leaves only. These are the main constituents responsible for the therapeutic value of the medicinal plants (Hussain *et al.,* 2011). According to Ayodele (2003) diverse use of plants in the treatment of wide variety of diseases are attributable to the presence of the phytochemicals. Phytochemicals are secondary metabolites produced and used by the plants for protection and repair the processes within the natural environment. Phenols, flavonoids and tannins are good antioxidant substances which have been reported to have anti-diarrhoeal activity also (Agbor *et al*., 2004) and prevent or control oxidative stress related disorders (Vinson *et al*., 1995).

Total flavonoids and phenolic content of leaves and roots of *L. mollis* in rutin and gallic acid equivalents are presented in Table 3. 0.017 and 0.013 mgRE/g extract, 458.91 and 704.50 mgGAE/g extract flavonoids and phenols were present in leaves and roots respectively. The flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996.). They are also effective antioxidants and show strong anticancer activities (Salah *et al.,* 1995; Del-Rio *et al.,* 1997; Okwu, 2004). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.,* 2007). They possesses biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis and cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.,* 2007). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Krings and Berger, 2001).

The results revealed the presence of medicinally important constituents such as flavonoids and phenol in the study plant. Further the plant could be considered for antioxidant, anticancer, immunomodulatory activities.

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Table 1. Extractive values of the methanolic leaf and root extracts of *Lagascea mollis.*

S.No.	Parts	Colour	Weight of crude extracts(g)/20g plant materials	% yield
1.	Leaves	Dark green	3.68	18.4
2.	Roots	Light brown	2.81	14.05

Table 2. Phytochemical screening of the methanolic leaf and root extracts of *Lagascea mollis.*

S.No.	Tests	Parts used	
		Leaves	Roots
1.	Alkaloids	+++	+++
2.	Flavonoids	+++	+++
3.	Glycosides	-	-
4.	Saponin	+++	-
5.	Tannin	-	-
6.	Steroids	-	-
7.	Terpenoids	++	+++
8.	Phenols	-	-

Table 3. Estimation of total flavonoids and phenolic content of methanolic leaf and root extracts of *Lagascea mollis.*

S. No	Parts	Total Flavonoids mg RE/g extract	Total Phenolics mg GAE/g extract
1.	Leaves	0.017±0.002	458.91±6.53
2.	Roots	0.013±0.005	704.50±20.40

GAE - Gallic acid equivalent, RE – Rutin equivalent.
Values are expressed as mean±SD (n=6).

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A COMPARATIVE STUDY OF ANTIOXIDANT POTENTIAL OF *GNAPHALIUM POLYCAULON,* TRADITIONAL INDIAN FOLK MEDICINAL PLANT

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ABSTRACT

The current study was undertaken to examine the antioxidant value of *Gnaphalium polycaulon* in different solvents. The fresh plant parts were collected from Kodanadu, The Nilgiri District, South India. Plant materials are washed, air dried and coarsely powdered by soxhlet apparatus for organic solvent extraction methanol, ethanol, hexane and water at 4°C. Then all the extracts obtained were subjected for antioxidant analysis using enzymic, non-enzymic and total antioxidant assays. All the methanolic extracts exhibited antioxidant activity significantly. The order of antioxidant value in *G.polycaulon* showed that the leaf, stem and flower. we reported that our finding provided support that the crude solvent plant extracts contain medicinally important free radicals scavenging compounds due to the strongly presence of phytoconstitutents. The study reveals that the consumption of the most valuable plant, *G.polycaulon* would exert several beneficial effects by virtue of their antioxidant activity in the traditional folk medicines for the treatment of different diseases.

Keywords: *Gnaphalium polycaulon*, medicinal plant, free radicals, antioxidant, $DPPH^{\bullet}$, $ABTS^{\bullet+}$

1. INTRODUCTION

Aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant and antimicrobial properties that can protect the human body against both cellular oxidation reactions and pathogens (Shanmugapriya and Thayumanavan, 2013). Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Bhore *et al.*, 2012). The use of plants for medicinal purposes and folk medical practices can be traced back to earlier civilization that is prevalent in rural and tribal villages (Badugu 2012). Traditional herbal medicine is an important component of primary health care system in developing countries for the revival of herbal plants (Shanmugapriya *et al.,* 2014). Medicinal plants have a global distribution although they are most abundant in the tropics. About 80% of the world population relies on herbal traditional medicine for their primary health care (Rubina and Qaiser, 2008).

Free radicals are highly reactive particles with an unpaired electron and are produced by radiation or as byproducts of metabolic processes (Shanmugapriya *et al.,* 2011). A serious imbalance between the production of free radicals and the antioxidant defense system is responsible for oxidative stress (Chakraborty and Shah, 2011). Dietary antioxidants protect the body against free radicals (Sudha, 2011). Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS), reactive nitrogen species (RNS). Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells prevent damage to lipids, proteins, enzymes, carbohydrates DNA (Shanmugapriya *et al.,* 2012). Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones (Mishra and Satpal, 2011).

Phytochemicals are natural and non-nutritive bioactive compounds produced by plants that act as protective agents against external stress and pathogenic attack (Chew *et al*., 2011). Flavonoids are capable of treating certain physiological disorder and prevent oxidative cell damage (Okwu, 2004). In addition, vitamin C, vitamin E and carotenoids, polyphenols (a wide class of components including phenolic acids, catechins, flavonols and anthocyanins), have shownstrong antioxidant capacity (Zhishen *et al*., 1999). DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. Chromatophore ABTS+ was formed by the reaction between ABTS and potassium persulphate and reduced to ABTS by the action of antioxidants available in the extracts (Mensor, 2001).

Gnaphalium polycaulon is a genus of flowering plants in the Asteraceae family of compositae type, worldwide distribution and is mostly found in temperate regions, although some are found on tropical mountains or in the subtropical

regions of the world (Shih and Ming, 2006). The entire plant is harvested during flowering and is used to make herbal and homeopathic remedies (Bhupendra *et al.,* 2008). Species in this genus are said to have anti-inflammatory, astringent, and antiseptic properties and are often prescribed as an herbal supplement for colds, flu, pneumonia, tonsillitis, larygitis, and congestion (Uniyal and Shiva, 2005).

Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury. Researchers revealed that the plant kingdom has not been exhausted based on the species of medicinal plants which are yet to be discovered. The investigations of natural antioxidants from medicinal plants are numerous. In current herbal drug scenario, plant derived antioxidants are gaining importance because of their potential health benefits, no toxicity and side effects over synthetic antioxidants (Jaina *et al*, 2011). Plants may contain a wide variety of free radical scavenging molecules based drugs/formulations used for the prevention of complex diseases (Jinu *et al.,* 2014). So, this unique medicinal plant was chosen for our present study with main objectives to highly remarkable investigates antioxidant potential.

2. MATERIALS AND METHODS

2.1. Chemicals required

All chemicals used for this study were high quality analytical grade reagents. The solvents such as ethanol, water and hexane were purchased from S.D. Fine Chemicals Pvt. Ltd, Sigma chemicals, Lobe chemicals, Merck Chemical Supplies, Nice Chemicals and Hi media. All other chemicals used for the study were obtained commercially and were of analytical grade.

2.2. Collection of plant material

The fresh leaves, stem and flower of *Gnaphalium polycaulon* plant were collected from Kodanadu near Kotagiri in The Nilgiri district, South India.

2.3. Extraction of Plant Material

The plant materials were washed, air dried and coarsely powdered. Forty grams of the powdered sample was extracted sequentially by using Soxhlet's extractor for 72h at a temperature not exceeding the boiling point of the solvent into 250ml of methanol, ethanol, hexane, and water for extract preparation. Resulting extracts was filtered using Whatman filter paper (No.1) and concentrated in vacuum to dryness using a Rotary evaporator. Powder was weighed and dissolved in the appropriate solvents used for extraction separately and stored at 4 ˚C for further use.

2.4. Antioxidant activity

2.4.1. Enzymic antioxidants

The enzymic antioxidants were analyzed in the fresh plant parts by standard methods. The assays were catalase (CAT) (Luck,1974), Peroxidase (POD) (Reddy *et al*., 1995), Glutathione S-Transferase (GST) (Habig *et al*., 1974) and Polyphenol Oxidase (PPO) (Esterbauer *et al.,*1977).

2.4.2. Non-enzymic antioxidants

The non-enzymic antioxidants were estimation out with standard methods such as Ascorbic acid (Sadasivam. and Manickam, 1997), Tocopherol (Sadasivam. and Manickam, 1997), Total Carotenoids and Lycopene (Zakaria *et al*., 1979), and Reduced Glutathione (Boyne and Ellman, 1972).

2.4.3. Free radical scavenging activity

2.4.3.1. DPPH$^{\bullet}$ scavenging assay

The scavenging ability of the natural antioxidants of the plant extracts towards the stable free radical DPPH were measured by the method of Mensor *et al.,* 2001. Each plant extracts (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH$^{\bullet}$ in methanol, without the plant extracts, served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer. The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity } \% = 100 - \frac{A_{518}\ (\text{Sample}) - A_{518}\ (\text{Blank})}{A_{518}\ (\text{Blank})} \times 100$$

2.4.3.2. ABTS$^{\bullet+}$ scavenging assay

The antioxidant effect of the plant extracts were studied using ABTS$^{\bullet+}$ (2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay according to the method of Shirwaikar *et al.,* 2006. ABTS radical cations (ABTS$^{\bullet+}$) were produced by reacting ABTS solution (7mM) with 2.45mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5ml) of the three different extracts were added to 0.3ml of ABTS$^{\bullet+}$ solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm in a spectrophotometer and the percent inhibition was calculated using the formula

$$\text{Scavenging activity } \% = 100 - \frac{A_{745}\ (\text{Sample}) - A_{745}\ (\text{Blank})}{A_{745}\ (\text{Blank})} \times 100$$

3. RESULTS AND DISCUSSION

Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of disease.

3.1. Antioxidant Activity

The enzymic antioxidants in the fresh plant parts were analyzed by the assay of catalase (CAT), peroxidase (POD), glutathione S-transferase (GST) and polyphenol oxidase (PPO). The results are tabulated in Table-1. In compared with all extracts, the values of methanolic leaf extract of each assay were found to be CAT (0.71), POD at 30 sec and 60 sec (0. 803 and 0.935), GST at 15 sec and 30 sec (0.69 and 0.68) and PPO (1.60) are expressed in U/mg protein. All assays reported that the fresh methanolic leaf extracts of *G.polycaulon* showed high enzymic antioxidants than other extracts.

3.2. Non-enzymic Antioxidant

The non-enzymic antioxidants were ascorbic acid, α-tocopherol, total carotenoids, lycopene, and reduced glutathione. The results were reported and tabulated (Table-2). In compared with all extracts, the values of methanolic leaf extract of each assay were found to be Ascorbic acid (0.614), Total Carotenoids and Lycopene at 450 and 503 nm (0. 631 and 0.582), Reduced Glutathione at 412 nm (0.621) and Tocopherol (0.017) in mg/g. The fresh methanolic leaf extracts of *G.polycaulon* showed high enzyme antioxidants than other extracts.

3.3. DPPH• scavenging activity

The antioxidant activity of different plant extracts was determined using methanol solution of DPPH reagent. DPPH is a very stable free radical. The effect of an antioxidant on DPPH radical scavenging is due to their hydrogen donating ability or radical scavenging activity (Shanmugapriya *et al.,* 2014). When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenylpicryl hydrazine with the loss of its violet color (Mensor *et al.,* 2001). The results of the DPPH radical scavenging activity of *G.polycaulon* showed that the fresh methanolic extract possesses very high percentage antioxidant activity of 84.5% at a concentration of 500 µg/ml than stem and flower in others solvents. The results shows that *G.polycaulon* plant extracts have hydrogen donors that scavenge the free radical DPPH•, with high Antioxidant activity in methanolic fresh leaf extract that was observed to be higher than standard (Table-3 and Figure-1).

The result of DPPH• scavenging activity assay in this study indicates that the plant was potently active (Yogesh *et al.,* 2011). The ability of this plant extract to scavenge DPPH• could also reflect its ability to inhibit the formation of $ABTS^{\bullet+}$ (Stephanie *et al.,* 2009). The DPPH• test provides information on the reactivity of the test compounds with a stable free radical. DPPH• gives a strong absorption band at 517 nm in visible region (Tyagi *et al.,* 2010). When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolorized as the color changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging power of the extract.

3.4. $ABTS^{\bullet+}$ radical scavenging activity

$ABTS^{\bullet+}$ radical, a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals. The $ABTS^{\bullet+}$ radical cation scavenging activity of the plant extracts were lesser in percentage when compared with that of DPPH• (Kriengsak *et al.,* 2006). Higher concentrations of the extracts were more effective in quenching free radicals in the system (Stephanie *et al.,* 2009). The highest percentage of antioxidant activity was 82.0% in 500 µg/ml concentration of methanolic extract (Table-4 and Figure-2). Among all the extracts, methanolic leaf extracts showed better results, fast and effective scavengers of the $ABTS^{\bullet+}$ radicals. Higher concentrations of the extracts were more effective in quenching free radicals in the system.

4. CONCLUSION

Antioxidant activity of medicinal plants plays a significantly part in postnatal recovery. Herbal medicines are not only providing traditional medicine but also promising for highly efficient novel bioactive molecules. The results revealed the presence of phytoconstituents in the plants showed that *G.polycaulon* plant is good source of antioxidant. The free scavenging activities of the *G.polycaulon* plants extracts are more effective, safe and non toxicity than synthetic antioxidant effects. Many medicinal plants lie unexplored or remain under explored. This finding supports the efficiency of selected plant as antioxidant additives or as nutritional supplements for human health in traditional medicine.

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Table 1. Enzymic antioxidant activity of fresh samples of *G.polycaulon.*

Enzymic antioxidant (U/mg protein)		OD (nm)	OD Value (nm) of fresh samples of *G.polycaulon*											
			Fresh leaf				Fresh stem				Fresh flower			
			M	E	H	W	M	E	H	W	M	E	H	W
CAT		240		0.67	0.65	0.70	0.64	0.60	0.58	0.61	0.62	0.60	0.51	0.56
POD	30 sec	430		0.792	0.787	0.799	0.781	0.756	0.721	0.772	0.685	0.601	0.628	0.673
	60 sec			0.889	0.853	0.922	0.801	0.786	0.691	0.790	0.699	0.678	0.656	0.687
GST	15 sec	340		0.53	0.49	0.61	0.57	0.51	0.47	0.50	0.49	0.32	0.34	0.44
	30 sec			0.51	0.48	0.60	0.56	0.49	0.42	0.48	0.51	0.48	0.43	0.49
PPO		495		1.54	1.52	1.56	1.56	1.41	1.39	1.42	1.36	1.33	1.42	1.33

Table 2. Non-Enzymic antioxidant activity of fresh samples of *G. polycaulon.*

Non-Enzymic antioxidant (mg/g)	OD (nm)	OD Value (nm) of fresh samples of *G.polycaulon*											
		Fresh leaf				Fresh stem				Fresh flower			
		M	E	H	W	M	E	H	W	M	E	H	W
Ascorbic acid	540	0.614	0.587	0.563	0.605	0.612	0.594	0.566	0.600	0.581	0.578	0.546	0.531
Total Carotenoids	450	0.631	0.598	0.584	0.613	0.629	0.581	0.572	0.617	0.574	0.566	0.552	0.572
and Lycopene	503	0.582	0.537	0.528	0.576	0.580	0.543	0.530	0.578	0.558	0.521	0.519	0.542
Reduced Glutathione	412	0.621	0.605	0.591	0.611	0.615	0.590	0.592	0.607	0.611	0.596	0.584	0.605
Tocopherol	460, 520	0.017	0.011	0.008	0.013	0.015	0.011	0.009	0.012	0.011	0.008	0.005	0.010

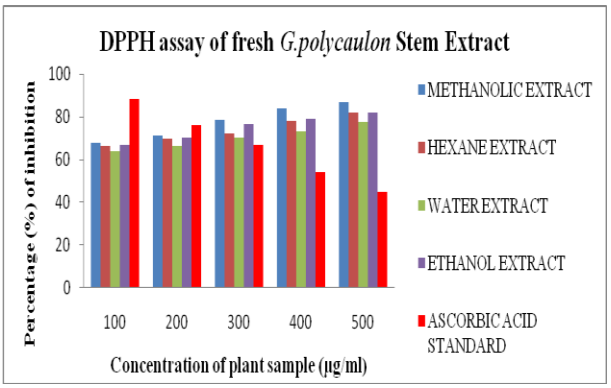
Table 3. DPPH$^{\cdot}$ scavenging activity of fresh samples of *G.polycaulon.*

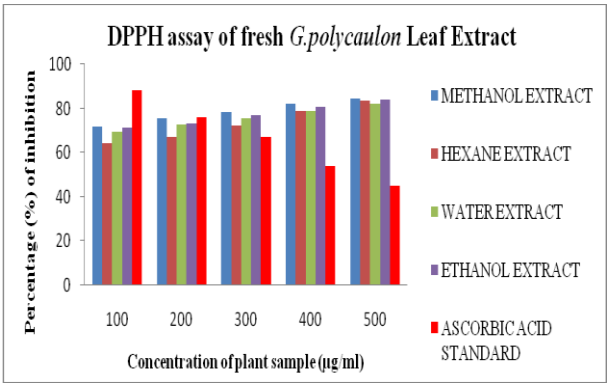
Plant samples	Concentration (µg/ml)	Percentage of Inhibition (%)				Standard Ascorbic acid
		DPPH assay				
		M	H	W	P.E	
Fresh Stem	100	67.9	66.2	64	66.7	88.30
	200	71	69.6	66.4	70.2	76.15
	300	78.6	72.3	70.1	76.4	67.03
	400	84	78.1	73.2	79.2	53.98
	500	87	82.1	77.5	82.1	45.11
Fresh Leaf	100	71.9	64.1	69.6	71.2	88.30
	200	75.7	67.3	72.7	73.3	76.15
	300	78.4	72.4	75.7	77.2	67.03
	400	82.3	79	78.8	80.7	53.98
	500	84.5	83.5	82.3	83.9	45.11
Fresh Flower	100	61.5	60.1	60	60.3	88.30
	200	68.7	65.2	64.2	64.4	76.15
	300	70.2	67.5	65.6	69.3	67.03
	400	72.7	71.8	71	72.4	53.98
	500	78	73.8	72.3	74.7	45.11

Table 4. ABTS$^{\cdot+}$ radical scavenging activity of fresh samples of *G. polycaulon.*

Plant samples	Concentration (µg/ml)	Percentage of Inhibition (%)				Standard Ascorbic acid
		ABTS assay				
		Methanol	Hexane	Water	Ethanol	
Fresh Stem	100	64.5	63.9	63.3	63.7	82.50
	200	67.5	67.3	66.8	66	71.03
	300	70.9	69.2	70.1	67.8	60.88
	400	73.9	71.4	71.3	69.2	51.08
	500	78	74.9	75.3	70.9	42.79
Fresh Leaf	100	65.4	61.4	65.1	64.8	82.50
	200	67.7	65.5	65.5	67.1	71.03
	300	71.9	68.7	70.6	69.6	60.88
	400	78.2	72.4	72.7	71.7	51.08
	500	82	77.6	74.8	73.7	42.79
Fresh Flower	100	60.3	59.6	58.8	56.1	82.50
	200	64.2	63	62.6	58.5	71.03
	300	65.7	65.1	63.5	61.6	60.88
	400	71	69.5	66.1	64	51.08
	500	72.5	72.1	68.2	66.4	42.79

Fig. 1 DPPH$^{\bullet}$ scavenging activity of fresh samples of *G. polycaulon*





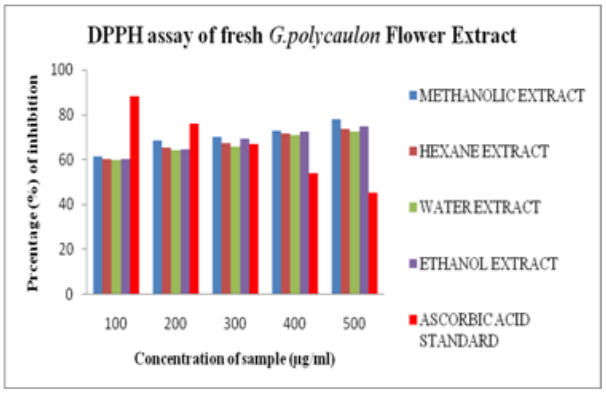
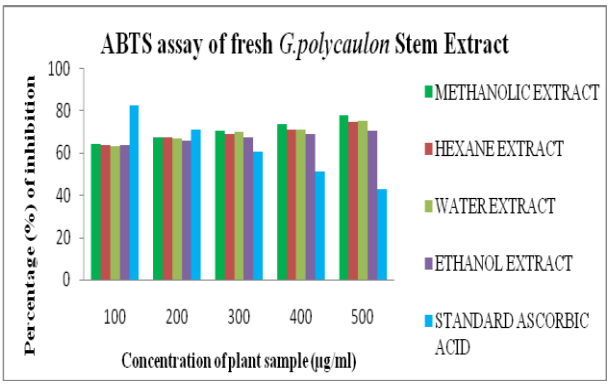
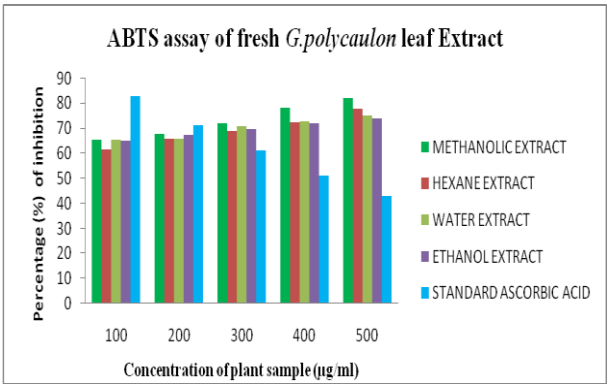
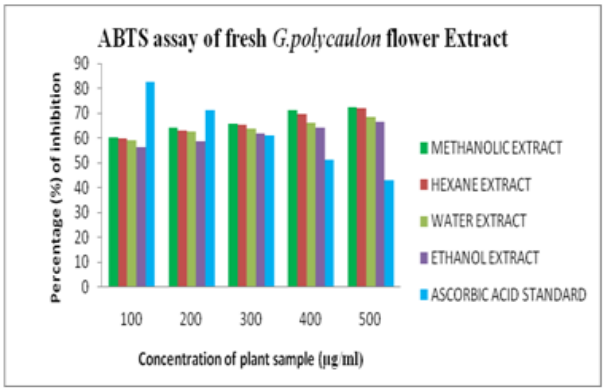


Fig. 2 ABTS$^{\bullet+}$ scavenging activity of fresh stem, leaf and flower samples of *G. polycaulon.*







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PHYSICO AND PHYTO-CHEMICAL ANALYSIS OF *PLUMBAGO ZEYLANICA* L. - A POTANT INDIAN HERB

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ABSTRACT

Medicinal plants are an integral component of alternative medical care. For millennia, Indian people traditionally played an important role in the management of biological resources and were custodians of related knowledge that they acquired through trial and error over centuries. India has a rich wealth of medicinal plants and the potential to accept the challenge to meet the global demand for them. In recent time, focus on plant research has increased all over the world and a large body evidence has collected to show immense potential of medicinal plants used in various traditional system. *Plumbago zeylanica* L. (Plumbaginaceae) is a tropical plant and the source for the well known ayurvedic drug 'chitrakah'. Different parts of this plant are widely used for variety of medicinal treatments. Especially used for appetite, indigestion, piles, worms, liver diseases and cancer treatments. The present study was therefore carried out to provide pharmacognostic details of root of *Plumbago zeylanica*. The physico-chemical analysis of ash content indicated the presence of inorganic matter and siliceous matter in the drug. The phytochemical analysis indicated the presence of alkaloids, phenols, flavonoids, tannins and terpenoids. Plumbagin is a naturally occurring yellow pigment, produced by member of plumbaginaceae that can be obtained from roots.

Keywords: Medicinal plants, traditional, ayurvedic, chitrakah, cancer, plumbagin

1. INTRODUCTION

The use of plants in ethnomedicine is increasing around the world. The World Health Organization (WHO) has reported that approximately 80% of the world's population currently uses herbal medicines (Kalidass *et al*., 2010). Herbal drugs are widely prescribed, even when their biological components are not known, as a result of their effectiveness, fewer side effects and relatively low cost (Kumar *et al*., 2009). The main source of drugs for Indian system of medicine, majority of the Indian population depends on phytomedicine for their primary health care in this modern scientific world (Thinakaran *et al*., 2009). In recent time, focus on plant research has increased all over the world and a large body evidence has collected to show immense potential of medicinal plants used in various traditional system. Silver and Bostian (1993) have documented the use of natural products as new antibacterial drugs. There is an urgent need to identify novel substances active towards highly resistant pathogens (Cragg *et al*., 1997). In an effort to discover new compounds, many research groups screen plant extracts to detect secondary metabolites with the relevant biological activities. In this regard, several simple bioassays have been developed for screening purposes (Hostettmann, 1991).

The present study was carried out on the phyto-chemical analysis of root extract of *Plumbago zeylanica* L. (Plumbaginaceae) which is a tropical shrub commonly distributed in forest of the Uttarakhand, India and cultivated in the gardens throughout India. The plant is commonly known as Ceylon Leadwort (English) and Chitramoolam (Tamil). *Plumbago zeylanica* is the source for the well known ayurvedic drug ' Chitrakah'. The whole plant, roots, powder of the root, leaves and stem-bark are widely used as medicinal herbs throughout Asia and Africa. In traditional herbal medicine, the root park of *Plumbago zeylanica* is used for treatment of different ailments such as parasitic diseases, scabies and ulcers (Olagunju *et al*., 2006), piles, diaarrhoea, skin diseases and leprosy (Uma *et al*., 1999), fever, rheumatism, intestinal parasites, anemia, swelling and scabies (Jeyachandran *et al*., 2009), antimicrobial (Ahmad *et al*., 2000), antioxidant and anti-inflammatory (Raimi and Oyedapo, 2009), antiplasmodial (Simonsen *et al*., 2001) and anti-insecticidal (Arunachalam *et al*., 2010).

2. MATERIALS AND METHODS

2.1. Plant Material

The root parts of *P. zeylanica* L. were collected from Kolinchamparai, Palakkad (Palghat), Kerala, India (Plate-1). Collected specimens were

carefully examined and identified with the help of regional floras (Kirithkar and Basu, 1980). Specimens were further confirmed with reference to herbarium sheet available in the Botanical Survey of India, Southern Circle, Coimbatore.

2.2. Preparation of extract

The roots were separated from the stalks and thoroughly but gently rinsed with tap water thrice to remove sands then oven dried completely at 27 to 30˚C for 1 week. The dried roots were ground to fine powder using a local grinder (Plate-2). 150 g of the powdered root was exhaustively extracted in alcohol, chloroform and petroleum ether with soxhlet extractor and then concentrated using rotary vacuum evaporator. 10 ml extract was measured out from the concentrate for phytochemical screening while the remaining extract was evaporated to complete dryness at 35˚C given a dark brown colour solid residue. The dried extract was stored in airtight container and placed in refrigerator.

2.3. Physico and Phyto-chemical screening

The macroscopic study of a medicinal plant was helpful in rapid identification of plant material and also plays an important role in standardization of drug. The fresh root was subjected to macroscopic studies which comprised of organoleptic characters viz., color, odour, appearance, taste, texture etc. Organoleptic characters (colour, odour, taste, texture) of root powder and the successive extracts were observed and recorded (Evans, 1983). Behaviour of plant powder with different chemical reagents (Kokoshi *et al*., 1958), Determination of moisture content (Anonymous, 1966) and Ash values (Evans, 1983) were observed and recorded. Preliminary phytochemical screening were carried out for alkaloids, phenolics, flavonoids, steroids, sterols, tannins, sugars and terpenoid (Harborne, 1984; Kokate *et al*., 1995).

3. RESULTS AND DISCUSSION

The organoleptic evaluation of the root powder of *P. zeylanica* showed characteristic odour and pungent taste. Upon drying and powdering the colour of the powder changed from light brown to reddish brown as shown in Table 1. The organoleptic characters such as colour, consistency and odour were noted in the successive root extracts (Table 2). The behaviour of root powder with different chemical reagents were observed and presented in Table 3. The physico-chemical characters of the sample were analysed and presented in Table 4. Loss on drying at 105˚ is determined since the presence of excess moisture is conducive to the promotion of mould and bacterial growth and subsequently to deterioration and spoilage of the drug (Ariyanathan *et al*., 2010; Pravin Sopan Borhade *et al*., 2014). The physico-chemical analysis indicated the 3.11% of ash content which is due to the presence of inorganic matter. Acid insoluble ash indicates the presence of more siliceous matter (0.96%) in the drug.

Plants are considered as bioreactors or biosynthetic laboratories as they synthesize wide range of characteristic therapeutically important molecules in the form of secondary metabolites. Thus, a systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and to establish a chemical profile of a crude drug for its proper evaluation (Pravin Sopan Borhade *et al*., 2014). The benzene soluble extractive reveals the presence of flavonoids, steroids, sterols, tannins, sugar and terpenoids. The chloroform soluble extract reveals the presence of steroids, sterols and terpenoids. The ethanol soluble extract reveals the presence of alkaloids, phenols, flavonoids and sugars. The water soluble extract reveals the presence of phenols, flavonoids and sugars. In the preliminary phytochemical analysis, *P. zeylanica* (Table 5) showed the presence of secondary metabolites like alkaloids, phenols, flavonoids, Tannins and Terpenoids (Dhale and Markandeya, 2011).

4. CONCLUSION

Preliminary phyto-chemical as well as various aspects of the sample were studied and described along with physico-chemical and microscopic studies in authentication, adulteration for quality control of raw drugs. Preliminary phyto-chemical analysis showed the presence of secondary metabolites like alkaloids, Phenols, flavonoids, tannins, and terpenoids. These studies will help in proper identification of the plant as a whole and its powder form for future studies.

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Table 1: Organoleptic characters of root powder of *P. zeylanica*

S.No.	Characters	Observations
1.	Colour	Reddish brown
2.	Texture	Fine smooth powder
3.	Taste	Pungent
4.	Odour	Characteristic smell

Table 2: Organoleptic characters of plant root successive extracts of *P. zeylanica*

S.No.	Extraction Medium	Colour	Consistency	Odour
1.	Alcohol	Light brown	Semi solid	Characteristic smell
2.	Chloroform	Brownish black	Semi solid	Characteristic smell
3.	Petroleum ether	Brownish black	Solid	Characteristic smell

Table 3: Behaviour of root powder of *P. zeylanica* with different chemical reagents

S.No.	Powder + Reagents used	Colour of the powder
1.	Powder as such	Pale brown
2.	Powder + Concentrated HCL	Wood brown
3.	Powder + Concentrated HNO_3	Yellowish
4.	Powder + Concentrated H_2SO_4	Greenish brown

Table 4: Physico-chemical and extractive values of root powder of *P. zeylanica*

S.No.	Physico-chemical properties	Values in percentage (%)
1.	Moisture content	8.18
2.	Total ash	3.11
3.	Acid insoluble ash	0.96
4.	Water soluble ash	2.27
5.	Alcohol-soluble extractive	12.83
5.	Crude fibre content	14.30

Table 5: Qualitative analysis of root extracts of *P. zeylanica*

S.No.	Phytochemical constituents	Ben	$CHCl_3$	E	H_2O
1.	Alkaloids	-	-	+	-
2.	Phenols	-	-	+	+
3.	Flavonoids	+	-	+	+
4.	Steroids, sterols	+	+	-	-
5.	Tannins	+	-	-	-
6.	Sugar	+	-	+	+
7.	Terpenoids	+	+	-	-

Note : '+', ' –' indicates the presence / absence of compounds. (Ben) Benzene (CHCl3), Chloroform, (E) Ethanol, (H2O) Water.

PLATE-I

Plumbago zeylanica – Habit

PLATE-II

Plumbago zeylanica – Root

Plumbago zeylanica – Root Powder

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Kongunadu Arts and Science College, Coimbatore

DIVERSITY OF BASIDIOMYCETES IN DODDANNI FOREST, THE NILGIRIES

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ABSTRACT

Studies on the taxonomy and diversity of macro fungi are gaining importance as many macro fungi are becoming extinct and facing threat of extinction because of habitat destruction. Present study deals with the diversity of macro fungi in Nilgiri Biosphere Region (NBR) of Western Ghats. Extensive surveys were conducted from June 2011 to February 2012, where collection, preservation and photo of macro fungal carried the genera like are *Boletus, Suillus, Leccinum, Tylopilus, Lactarius, Russula, Amanita, Tricholoma, Stropharia, Entoloma, Cortinarius, Scleroderma* and *Agrocybe* were identified. A total of 20 species were collected belonging to 10 genera. Also the temperature, rainfall and the relative humidity of the study area was noted periodically. This preliminary study shows that the forest is very rich in mushroom diversity.

Key-Words: Nilgiri Biosphere Region (NBR), Maccro fungi, Diversity, climatic data.

1. INTRODUCTION

Fungi are a very large, diverse group of living organisms found in nearly all ecosystems. Fungi which form an important component of the forest ecosystem have been largely neglected in any of the biodiversity studies of a given area and play key roles in all ecosystems as saprophytic, pathogens and symbionts (Mueller *et al.,* 2007; Schmit and Mueller, 2007). The present correspondence is an attempt to give a broad picture of the biodiversity of a particular group of fungi, viz. members of order Agaricales, Class Basidiomycetes in the NBR. Natarajan *et al.* (2005) present a broad picture of the biodiversity of members of Agaricales, a particular group of fungi in the class Basidiomycetes, in the Nilgiri Biosphere Region (NBR) of Western Ghats. There are a large number of undescribed species present in this region.

Currently, there is little knowledge of mycodiversity and also a deficiency of systematic taxonomic work achieved in NBR. The purpose of the present survey was to identify the mushrooms up to genus and species level, to record and compare the diversity of mushrooms to other areas and to bring awareness among people to conserve mushrooms. Basidiomycetes fungi have been known to synthesize a vast array of secondary metabolites that possess beneficial biological activities (Dong-Ze *et al*., 2008). The medicinal properties of basidiomycetae mushrooms are frequently described in ancient cultures and a few have been developed into pharmacological applications today. In Asian cultures, the holocarp of several genera are eaten, included as a garnish, or boiled as teas and applied as therapies for a variety of human ailments ranging from the common cold to cure for certain forms of cancers (Loreto Robles-Hernandez, 2008).

A total number of 223 species belonging to 61 genera have so far been reported from the Nilgiri Biosphere Reserve (Natarajan, 2005). Many basidiomycetae fungi serve an important ecological role as wood decomposers. Our main objectives of this study is to target the diversity of macro fungi, in Nilgiri Biosphere Region (NBR) of Western Ghats, India.

2. MATERIALS AND METHODS

2.1. Study area

The present study area Doddani is located in a distance of 15 km from Kothagiri of Nilgiri District at a latitude of 10°24'N and a longitude of 63°36'E at an altitude of 1900 ms above msl with 170ha. The study area is situated at sub-tropical forest region.

2.2. Climatic data

The climatic data of the study area for the study period of 9 months from June, 2011 to February, 2012 is given in table 1. The temperature during the study period was varied between 19 and 23°C. The rainfall during the study period was about 180 mm. The relative humidity was existing between 93 and 15 percent.

3. RESULTS AND DISSCUSSION

The climatic factor of the study area is given in Table 1. Maximum and minimum temperature, relative humidity and rainfall were observed from June 2011 to February 2012. The highest maximum temperature was 25°C during September – December 2011. The highest minimum temperature

was noted as 20°C on June, 2011 and the lowest minimum was 10°C from July – December 2011. The average annual rainfall during the study period June, 2011 was 157.44. The precipitation was mainly through south-west (June-August) and north-east (October - November) monsoon. The relative humidity during study period ranged between 70 and 95%. Velocity of wind was moderate.

Table 1: Climatic data of the study area.

Year and month	Temperature °C		Rainfall (mm)	Relative humidity (%)
	Max	Min		
		2011		
June	22	20	119	75
July	21	10	161	81
August	22	10	126	70
September	20	10	179	80
October	20	10	263	93
November	20	10	244	91
December	20	10	89	90
		2012		
January	22	15	110	90
February	25	15	126	95

Table 2: Edible status of collected Basidiomycetes.

Family	Genus	No. of Species
Boletaceae	*Boletus*	2
Suillaceae	*Suillus*	2
	Leccinum	1
	Tylopilus	1
Russulaceae	*Lactarius*	2
	Russula	2
Amanitaceae	*Amanita*	3
Tricholomataceae	*Tricholoma*	1
Strophariaceae	*Stropharia*	2
Entolomataceae	*Entoloma*	1
Cortinariaceae	*Cortinarius*	1
Sclerodermataceae	*Scleroderma*	2
Bolbitiaceae	*Agrocybe*	1

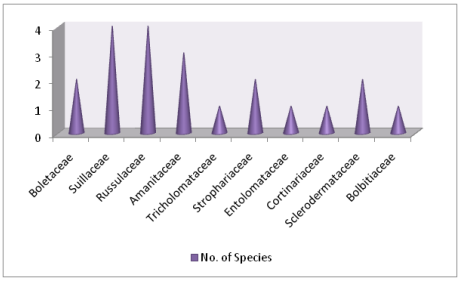

Table 3: Distribution of different species and family wise distribution of mushrooms of subdivition Basidiomycotina

Sl.No.	Family	Species	Total
1.	Boletaceae	*Boletus edulis* , *Boletus pulverulentus*	2
2.	Suillaceae	*Suillus americanus*, *Suillus neoalbidipes*, *Leccinum fibrillosum*, *Tylopilus cyaneotinctus*	4
3.	Russulaceae	*Lactarius lignyotellus*, *Lactarius piperatus*, *Russula fragrantissima*, *Russula ventricosipes*	4
4.	Amanitaceae	*Amanita flavoconia*, *Amanita muscaria*	2
5.	Tricholomataceae	*Tricholoma caligatum*	1
6.	Strophariaceae	*Stropharia hardii*, *Stropharia rugosoannulata*	2
7.	Entolomataceae	*Entoloma bloxamii*	1
8.	Cortinariaceae	*Cortinarius malicorius*	1
9.	Sclerodermataceae	*Scleroderma citrinum*, *Scleroderma michiganense*	2
10.	Bolbitiaceae	*Agrocybe paludosa*	1

The mycofloristic survey of Doddanni forest has several interesting mushrooms belongs to the Class Basidiomycetes. Based on Alexopolous and Mims system of classification, the collected mushrooms are coming under the Class Basidiomycetes, Order Agaricales, Lycoperdales and Phallales. Among these 3 orders, majority of the members are coming under Agaricales, which is followed by Lycorperdales and Phallales. The collected mushrooms are listed (Table-3).

The distribution of mushrooms in forest is higher than the grasslands because of the high nutrient contents. The occurrence of mushrooms is more in rainy season than the summer season (Ramsbottom, 1967). Among the 20 mushroom species, 8 edible belonging to the family Russulaceae, Suillaceae and Boletaceae. The family Russulaceae having 4 edible mushrooms, whereas in Suillaceae 2 edible mushrooms were collected. The same *Agaricus* species were collected as wild form in two localities of Himachal Pradesh (Upadhyay, *et al.,*2004). During the collected Basidiomycetes, the

families like Boletaceae have 4 species and Russulaceae with 4 species, whereas Amanitaceae and Sclerodermataceae are with 2 species each in Doddanni forest.

During the course of mycofloristic survey of Doddanni forest, a high diversity of macrofungi, were encountered. Among the fungus *Amanita flavoconia* an edible mushroom abundantly present in the study area. Some species of *Amanita* were reported Sri Lanka, India, China, South Korea, Japan, U.S.A. (Vrinda *et al.,* 2005). The other species *A.hemibapha* first described from Sri Lanka is a tropical relative of *A.caesarea. A.muscaria,* poison mushroom also collected in the study area.

During the survey genus *lactarius* sp. Of the family Russulaceae found more in different parts of the study area. It is also macrofungi having some interesting features. The same genus also recorded in North Western Himalaya region by (Das and Sharma, 2004).

Murali *et al.* (2007) studied foliar fungal endophytes of fifteen tree species from tropical dry thorn forest and tropical dry deciduous forest in Madumalai Wildlife Sanctuary, Nilgiri Biosphere Reserve, Southern India.

4. CONCLUSION

The fungi are an important component of the forest and grassland ecosystem which has been largely neglected by many biodiversity studies. So the present study has undertaken the biodiversity of the Basidiomycetes in the particular area of the Nilgiri district. This study indicates that, large number of fungi of this group occurs in the NBR of Tamil nadu and Kerala.

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PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL SCREENING OF THE LEAVES OF *KEDROSTIS FOETIDISSIMA* (JACQ.)COGN.

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ABSTRACT

The objective of the study is to cover the pharmacognostical and preliminary phytochemical screening of traditional medicinal plant, *Kedrostis foetidissima* belonging to the family Cucurbitaceae. This study includes organoleptic characters, physico-chemical analysis, fluorescence analysis, behaviour of leaf powder with different chemical reagents, phytochemical extraction, extractive yield and qualitative phytochemical screening. The preliminary screening revealed the presence of alkaloids, flavonoids, steroids, tannins, phenolics, glycosides, carbohydrates, proteins and aminoacids which explains that the plant must have valuable medicinal properties and so it can be explored.

Keywords: *Kedrostis foetidissima,* pharmacognostical and preliminary phytochemical screening.

1. INTRODUCTION

Medicinal plants provide an excellent source for valuable drugs. They have curative properties due to the presence of various secondary metabolites like phenolics, tannins, flavonoids, steroids, terpenes etc. Pharmacognostic study is the preliminary step in the standardization of crude drugs. It deals with the authentication and quality assessment of crude plant and herbal material based on macroscopic and microscopic characters (Heinrich, 2000). Evaluation of plant materials and their derived products are important in the field of discovery of phytopharmaceuticals (Sethiya *et al.,* 2010).

Kedrostis foetidissima is a traditional medicinal plant belongs to the family Cucurbitaceae. The fresh leaves are given orally to treat cattle suffering from both pasture and frothy bloats. (Kokwaro and John, 2009). Fruits are given internally in case of colic and abdominal pain. Roots externally applied with bark of *Pittosporum floribundum* for the treatment of piles. (Pullaiah, 2006). Crude leaf juice is given orally to treat common cold in children (Karuppusamy, 2007). The Zay people in Ethiopia were used this whole plant for curing the chest pain in human beings (Giday, 2001).

The present work deals with the pharmacognostic evaluation of the leaf material of *K. foetidissima* and establishment of its quality parameters, including phytochemical evaluation.

2. MATERIALS AND METHODS

2.1. Procurement and preparation of plant material

Fresh leaves of *Kedrostis foetidissima* were harvested from the surrounding areas of Coimbatore district, Tamil Nadu, India. The authenticity of the selected plant material was duly identified and confirmed by comparison with reference specimens preserved in the herbarium at Botanical Survey of India, Southern Circle, Coimbatore. The collected leaves were washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottle.

2.2. Pharmacognostic studies

2.2.1. Organoleptic evaluation

The plant powder was tested for colour, texture, odour and taste by following the method adopted by Jackson and Snowdown (1968).

2.2.2. Physicochemical parameters

The physicochemical parameters like moisture content, total ash content, acid insoluble and water soluble ash contents were determined as per WHO guidelines and using standard procedures (Trease and Evans, 1983; WHO, 2002).

2.2.3. Fluorescence analysis

The fluorescence properties of various extracts were studied in visible and ultra violet light (Kokoshi *et al.,* 1958).

2.2.4. Behaviour of drug powder

The powdered leaf samples were treated with different reagents and the colour developed was observed under room condition (Brain and Turner, 1975).

2.3. Phytochemical studies

2.3.1. Extractive yield

The air dried leaves were exhaustively extracted with successive solvent extraction using soxhlet apparatus viz., petroleum ether, benzene, chloroform, ethyl acetate, ethanol and hot water was performed as per Indian Pharmacopoeia (Peach and Tracey, 1955). The extracts were filtered and concentrated to dryness under reduced pressure using rotary vacuum evaporator (RE 300; Yamato, Japan), Lyophilized (4KBTXL – 75; Vir Tis Benchtopk, New York, USA) to remove traces of water molecules and their extractive yield percentage was calculated.

2.3.2. Qualitative phytochemical evaluation

Phytochemical screening for crude solvent extracts were carried out and their bioactive compounds were determined using standard methods (Brain and Turner, 1975; Trease and Evans, 1983; Harborne, 1984).

3. RESULTS AND DISCUSSION

Pharmacognostic studies generally encompass organoleptic characters, physicochemical analysis, fluorescence analysis and behaviour of leaf powder with different chemical reagents, all leading to the proper identification and authentification of the medicinal plants under study (Trease and Evans, 1985). In the present study, the organoleptic characters of the leaf powders (Table 1) showed that it is pale green in colour, coarsely powdered, bitter taste and having characteristic odour.

Table 1. Organoleptic characters of *Kedrostis foetidissima* leaf powder

S.No	Character	Observation
1.	Colour	Pale green
2.	Texture	Coarse powder
3.	Taste	Bitter
4.	Odour	Characteristic odour

The data on physico chemical characters of *K. foetidissima* leaves were presented in Table 2 and the leaves exhibited more moisture content of 87%. The total ash content was observed to be 9.22%. However, acid insoluble ash recorded higher value of 3.10% than the water soluble ash (0.34%). Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. The total ash values are used to determine quality and purity of crude drug. It indicates the presence of various impurities like carbonate, oxalate and silicate. The acid insoluble ash measures the amount of silica, especially sand which indicates the presence of earthy materials in the sample. The water soluble ash is used to estimate the amount of inorganic compounds present in drugs (Thomas *et al.,* 2008; Vaghasiya *et al.,* 2008; Dave *et al.,* 2010)

Table 2. Physico-chemical analysis of *Kedrostis foetidissima* leaves

S.No	Parameter	Percentage
1.	Moisture content	87
2.	Total ash	9.22
3.	Acid insoluble ash	3.10
4.	Water soluble ash	0.34

Extractive yield percentage and fluorescence analysis for different solvent extracts of *K. foetidissima* leaves were calculated (Table 3). The hot water and ethanol extracts registered maximum yield of 10.18 and 8.7% respectively. This kind of study helps in identifying the chemical entities favouring high percentage yield of the extraction (Kokate, 1995). The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in the estimation of specific constituents soluble in particular solvent (Thomas *et al.,* 2008). The fluorescence analysis was made both in ordinary and UV lights. This would offer guidance for further identification and confirmation of this plant.

Table 3. Extractive yield, fluorescence analysis of *Kedrostis foetidissima* leaf extracts under ordinary and UV lights.

S.No	Solvents	Extractive yield (%)	Colour by fluorescence analysis	
			Ordinary light	UV light
1.	Petroleum ether	3.7	Yellowish green	Parrot green
2.	Benzene	1.7	Brownish	Flourescent greenish brown
3.	Chloroform	0.8	Dark brown	Greenish black
4.	Ethylacetate	1.1	Brownish green	Greenish brown
5.	Ethanol	8.5	Golden brown	Fluorescent greenish brown
6.	Hot water (by maceration)	10.18	Light green	Parrot green

The behavioural patterns of powdered leaf of *K. foetidissima* with different chemical reagents were examined and tabulated (Table 4). These chemical tests showed the path for isolation of different active constituents present in this plant.

Table 4. Behaviour of *Kedrostis foetidissima* leaf powder with different chemical reagents.

Treatment	Colour observed
Powder as such	Pale green
Powder + Nitric acid	Wood brown
Powder + H_2So_4	Reddish brown
Powder + HCl	Dark green
Powder + Glacial acetic acid	Yellow green
Powder + Ferric chloride	Greenish brown
Powder + Iodine solution	Pale green
Powder + Ammonium solution	Yellowish green
Powder + Lead acetate	Pale green

All the extracts were subjected to preliminary phytochemical screening (Table 5) in that alkaloids and flavonoids were present only in the chloroform, ethyl acetate, ethanol and hot water extracts. Phenolic compounds and carbohydrates were found to be present only in the chloroform and ethyl acetate extract respectively. Petroleum ether extract provides positive results for only steroids whereas the benzene extracts showed the positive results for steroids, proteins and aminoacids. The triterpenoids, saponins and volatile oils were completely absent in all the extracts tested. Different solvents have been reported to have different capacity to extract phytoconstituents according to their solubility or polarity and most of the compounds are dissolving better in alcoholic solvents than in water (Marjorie, 1999).

Table 5. Qualitative phytochemical evaluation of *Kedrostis foetidissima* leaf extracts.

Test	Observation					
	PE	B	C	EA	E	HW
Alkaloids	-	-	+	+	+	+
Flavonoids	-	-	+	+	+	+
Steroids & Sterols	+	+	+	+	-	-
Terpenoids	-	-	-	-	-	-
Tannin	-	-	+	+	-	-
Phenolics	-	-	+	-	-	-
Glycosides	-	-	-	+	+	-
Saponins	-	-	-	-	-	-
Volatile oil	-	-	-	-	-	-
Carbohydrates	-	-	-	+	-	-
Proteins and aminoacid	-	+	+	-	-	-

PE-Petroleum Ether, B-Benzene, C-Chloroform, EA-Ethylacetate, E-Ethanol, HW-Hot water

Herbs have been an essential factor in health care throughout the ages and in all cultures. They are prepared in a number of ways to extract their active ingredients for internal and external uses. The standardization of a crude drug is an integral part of establishing its absolute identity.

3. CONCLUSION

In conclusion, the preliminary phytochemical evaluation indicates that *K. foetidissima* has the potential to generate novel metabolites. Therefore the bioactive compounds found in this species await a major breakthrough for the wellbeing of individuals after toxicological investigations.

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ETHNOMEDICINAL STUDIES ON RIVER STRETCH CAUVERY BASIN OF NANJAI EDAYAR AND KUCHIPALAYAM, NAMAKKAL DISTRICT, TAMIL NADU

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ABSTRACT

The study area covers about 15 km stretch in the perennial river bed, Cauvery which supports semi-evergreen to dry deciduous types of forests. Ethnomedicinal information on 26 plant species was recorded during the extensive field survey carried out in this stretch during 2011-2013. The information covers botanical names, vernacular names, family, plant parts used and the mode of usage.

Key words: Cauvery river basin; Medicinal plants; Traditional uses.

1. INTRODUCTION

Ethnobotanical studies carried out by varies workers have recorded that the tribal communities of India use about 7500 species of plants for a variety of medicinal purposes (Anonymous, 1994). In India, about 2500 plant species belonging to more than 1000 genera are used by traditional herbal healers and about 500 plant species are used by 159 different pharmaceutical companies (Chandel *et al.*, 1996; Vibhuti *et al.,* 2009). The large bulk of traditional knowledge of ethnic and rural people of India is handed down to generation through word of mouth and is extensively used for the treatment of common diseases and conditions (Ekka *et al.,* 2007). Primitive and village people of Namakkal district have been using several plants for combating disease from centuries and are found wide acceptance in traditional medicinal use. By the proper identification and documentation of such plants which are used in local treatment can help in developing easily available materials for the treatment of varies diseases as well as raising the health standards of the village people.

2. Study area

The Cauvery river stretch between Nanjai edayar and Kuchipalayam areas of Namakkal district Tamilnadu is situated at 11°04' L and 78°03' E . The altitude is 130.45 above MSL. The soil is mostly sandy with slightly acidic pH. The local public living in the adjoining villages in addition to western medicine also using herbal medicines for their day to day life. The present study was undertaken to document the traditional uses of medicinal plants available between Nanjai edayar and Kuchipalayam villages of Cauvery river basin in Tamilnadu. Grasslands, man-made plantation and semi-evergreen to dry deciduous types of forests are the common vegetations in this belt.

2. MATERIALS AND METHODS

The present study work was carried out in the study areas described during the period of 2011-2013. The forest areas and other vegetation were sampled for species identification near the villages *viz.,* Kuchippalayam, Velur, Nanjai edayar, Sengappalli and Palappatti.

A field survey was done in these villages for the plant based traditional medicinal practices and their uses. It consists of information interviewing of the villager's through the prepared questionnaire, who generally depend upon forests for their food and health and other needs of day to day life.

The identified and collected plant samples were arranged and documented according to their binomial. The traditional and the modern processing techniques of the medicinal plant species are enquired and briefed well. The total respondents were 4-6 in numbers per village. The species of medical importance were identified with the help of 'The Flora of Presidency of Madras' (Gamble, 1915-1935), The Flora of Tamilnadu Carnatic (Mathew 1983) and Indian Medicinal Plants (Kirtikar & Basu, 2003). Voucher specimens of the plants collected during the study were deposited in the Herbarium of the Department of Botany, Kandaswami Kandar's College, Velur, Namakkal, Tamilnadu.

3. RESULTS AND DISCUSSION

According to a report of World Health Organization, over ¾ of world population relies on the use of traditional medicine of plant origin (Rai *et al*., 2000). The traditional medicine has a long history and wide acceptability. Therefore,

documentation of information on indigenous knowledge and practices will help in conserving the traditional knowledge (Gani, 2003). The present enumeration shows the occurrence of 26 plant species of 25 genera belonging to 21 families (Table. 1). The study provides comprehensive information on the indigenous uses and traditional practices of the plants used in household remedies. All the 26 species documented were known to have intense use for the local public in the study villages. Species like *Abrus precatorius, Dendrophthoe falcata* and *Heliotropium indicum* are being used for wound healing purposes. Interestingly the species, *Catharanthus roseu*s is used for its anticancer property. Similarly the species, *Gymnema sylvestre* is used for the treatment of diabetics and *Zizypus jujuba* is used for the treatment of jaundice. The species, *Ocimum scantum* is having mosquitosidal property. The two species, *Cassia tora* and *Terminalia catappa* are prescribed for skin diseases and the species, *Cassia occidentalis* is used for the treatment of eye problems.

The study revealed that the Cauvery stretch between the Nanjai edayar and Kuchipalayam contains the vegetation rich in medicinal species which indicates the potentiality of this region in terms of traditional medicinal flora wealth. Therefore, sustainable utilization and effective conservation are supported to utilize and protect the species.

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Table 1: List of medicinal plants and their uses by villager's of Nanjai edayar and kuchipalayam villages of Cauvery basin.

Botanical name	Local name	Family	Parts used	Ethnomedicinal uses
Abrus precatorius L.	Gundumani	Fabaceae	Leaves, Roots, Seeds	*Fresh or dry leaes are ground and the infusion mixed with water is given orally once a day for three for menorrhoea.* Leaves of the white seed variety mixed with garlic, ground and the paste is given thrice a days for treating leucorrhoea. *The roots are also used to cure leucoderma, itching and wounds. *Seeds are poisonous, used by the locals to avoid pregnancy.
Acalypha indica L.	Kuppaimeni	Euphorbiaceae	Leaves	*Fresh leaves mixed with black pepper, garlic and long pepper, ground in water and the infusion given orally 3-6 times a day for the treatment of post-natal body pains.
Aegle marmelos L.	Vilvam	Rutaceae	Fruits	*The fruit is used in chronic diarrhoea and dysentery and said to at as a tonic for heart and brain.
Annona squamosa L.	Seethapazham	Annonaceae	Bark, Leaves, Roots, Fruits	*The bark is used in diarrhoea. *The root is employed in depression and spinal diseases, in asthma and fever. *The cursed leaves are reported to be applied to the nostrils in hysteria and fits. *The fruit possesses astringent, cooling, anti-scorbutic, and febrifugal properties. *Fruits is a source of vitamin C.
Alternanthera sessilis(L.) DC.	Ponnaankanni	Amaranthaceae	Herb	*The herb is used as a galactogogue, cholagogue, abortifacient and febrifuge. It is also used as a indigestion. *The whole plant is bitter, sweet, cooling and constipating. It is used to treat burning sensation, diarrhoea, leprosy, skin diseases, night blindness, dyspepsia and fever.
Aloe vera(L.) Burm.f.	Katarezhai	Liliaceae	Leaves and Roots	*Leaf pulp used to treat liver disorders, rheumatism, skin disorders, Vanishing cream, emollient, intestinal worms. *The leaf-gel has demand in cosmetic industry abroad and is also used to treat radiation burns.
Azadirachta indica A. Juss.	Vembu	Meliaceae	Bark, Leaves, seeds	*Neem oil has been found to slow down the growth of HIV-virus which causes AIDS. *The emulsified oil is used to control rust on beans and powdery mildew on many ornamental plants. *A handful of leaves, crushed and flattened, will make an excellent poultice for boils and sores; its action is stimulant and antiseptic.
Bacopa monnieri (L.) Pennel	Brahmi	Scrophulariaceae	Entire plant	*The entire plant constitutes the well-known drug Brahmi. It is used in the indigenous systems of medicine for the treatment of asthma, hoarseness, insanity, epilepsy, and as a potent nerve tonic, cardiotonic and diuretic. It is also used as a laxative, in several skin diseases and in bronchitis.

Calophyllum inophyllum L.	Punnai	Clusiaceae	Bark, Leaves, seeds	*A paste of the kernels is applied to relieve painful joints. *The refined oil is intramuscularly injected to alleviate pain in leprosy.*The oil cures scabies and other coetaneous diseases. *The pounded bark is applied in orchitis and its juice is taken as purgative. *The leaves are employed for inhalation in migraine and vertigo.
Calotropis gigantea (L.) R.Br	Erukku	Asclepiadaceae	Bark	*The root bark constitutes the drug. The powdered root bark gives relief in diarrhoea and dysentery. It is also given in cough and asthma, and as a febrifuge.
Cardiospermum halicacabum L.	Mudakkattrran	Sapindaceae	Entire plant	*The herb is diuretic, stomachic and rubefacient. It is used in rheumatism, lumbago, nervous diseases, as a demulcent in orchitis and dropsy. *The root is mucilaginous and considered effective in didymitis and early stages of hydrocele, and is used in asthma and colic.
Cassia occidentalis L.	Nattam-takarai	Caesalpiniaceae	Entire plant	*All parts of the plant possess purgative, tonic, febrifugal, expectorant and diuretic properties. *The plant is used to cure sore eyes, haematuria, rheumatism, typhoid, asthma and disorders of hemoglobin. *The herb forms an ingredient of the patented indigenous herbal drug "Liv-52", which shows marked effect in the early cases of hepatic cirrhosis having ciatorrhoea.
Cassia tora L.	Senavu	Caesalpiniaceae	Leaves ,Roots	*Both leaves and seeds constitute a valuable remedy in skin diseases, chiefly for ringworm and itch. *The weed is used in various Gold Coast medicines, chiefly as a purgative. *The root is not an antidote to either snake-venom or scorpion-venom.
*Catharanthus roseu*s (L.) G.Don.	Sudukattu mallikai	Apocynaceae	Entire plant	*The roots, which form the drug, contain more than 80 alkaloids of which Indole-Indoline dimers are pharmacologically very important. *Vinca alkaloids received great attention due to anticancer potential. Vincristine and Vinblastine are used as anticancer drugs especially for curing leukemia (blood cancer).
Ceiba pentandra (L.) Gaertn.	Ilavam	Bombacaceae	Fruits, Roots	*The roots are stimulant, tonic, diuretic, emetic and antispasmodic; they have hypoglycemic effect and are useful in diabetes, dysentery and gonorrhoea. *The unripe pods are astringent and demulcent, useful in vertigo and migraine. *The gum is given in bowel complaints and gonorrhoea.
Datura innoxia Mill.	Umathai	Solanaceae	Seeds	*As anesthetic in surgery and child birth, in ophthalmology and prevention of motion sickness.
Dendrophthoe falcata (L.f.) Etting	Pulluruvi	Loranthaceae	Bark	*The bark is astringent and narcotic, and is used for wounds and menstrual troubles and also as a remedy for asthma and mania. *The

				plant is cooling, aphrodisiac and diuretic and is useful in pulmonary tuberculosis, asthma, menstrual disorders, swellings, wounds and ulcers.
Euphorbia hirta L.	Amam patchaiarisi	Euphorbiaceae	Entire plant	*The entire plant, collected is flowering and fruiting stage and dried, constitutes the drug. It is useful in removing worms in children, in bowel complaints, asthma and cough. *It promotes formation and flow of milk in Woman; it is also useful in gonorrhoea and other urinogenitary complaints. The roots of the plant stop vomiting.
Gloriosa superba L.	Kanthal malar	Liliaceae	Seeds, Roots	*The tubers are regarded as tonic, somatic and antheimintic when taken in doses of 5-10 grains; in larger, doses they are intensely poisonous. Root tubers and seeds rich source of colchicine. *Colchicine is used in the treatment of cancer.
Gymnema sylvestre (Retz.) R.Br. Ex Schult.	Ciru kurinjan	Asclepiadaceae	Leaves, Roots	*The leaves used for treating diabetes. The plant is stomachic, stimulant, laxative and diuretic. The roots are emetic and expectorant.
Heliotropium indicum L.	Nakkipoo	Boraginaceae	Leaves	*The leaf juice is used as an application to wounds, sores, boils, gum-boils and to repel pimples on the face; boiled with castor oil it is applied to bites of scorpions, insects and reptiles. It is also employed locally in the kind of ophthalmic in which the tarsus is inflamed or excoriated.
Mangifera indica L.	Mampazham	Anacardiaceae	Leaves, Fruits	*Decoction of the leaves is used in fever, diarrhoea and toothache. *The dried mango peel can be used as a fuel for biogas plants. *The bio gas plant effluents are then used as a substitute for conventional fish feed.
*Ocimum sanctum*L.	Tulasi	Lamiaceae	Leaves, Seeds, Roots	*The plants drive away mosquitoes. It is useful in avarity of diseases. *Leaves ground with water are applied on bed boils. *Powder of the root rubbed slightly on a scorpion bite will give relief from pain. It basil root is taken, 1gm weight, at eve, increases the vital fluid and will bestow retentive virtue. Nerve weakness may be cured by it.
Pergularia daemia L.	Vealipparutthi	Asclepiadaceae	Entire plant	*The juice from the tender vegetative parts is used to cure toothache by the village people of Namakkal district.
Terminalia catappa L.	Kadookkai	Combretaceae	Leaves	*Leaf juice of young leaves is used to prepare an ointment for skin diseases.
Zizypus jujuba L.	Elandai	Rhamanaceae	Bark, Roots	The fruits juice has cooling effect, and its syrup is used to cure jaundice and other abdominal diseases. Bark is used for Teeth diseases and flu.

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INVASIVE ALIEN WEEDS AND THEIR ETHNO-BOTANICAL IMPORTANCE OF VIJAYAMANGALAM VILLAGE, ERODE DISTRICT

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ABSTRACT

The present study deals with comprehensive list of Invasive alien plants of Vijayamangalam village, Perundurai taluk, Erode district with background information on family, habit and nativity. A total of 50 invasive alien species under 40 genera, belonging to 18 families have been recorded. While in life form analysis, the herbs (40 species) are dominant, followed by shrubs (6 species), Climber (2 species), Trees (2 species).

Key words: Invasive alien species, Vijayamangalam, family, nativity.

1. INTRODUCTION

Alien species are non-native or exotic organisms that occur outside their natural adapted ranges and dispersal potential. Many alien species support our farming and forestry systems in a big way. However, some of the alien species become invasive when they are introduced deliberately or unintentionally outside their natural habitats into new areas where they express the capability to establish, invade and out compete native species. International Union for Conservation of Nature and Natural Resources (IUCN) defines Alien Invasive Species as an alien species which becomes established in natural or seminatural ecosystems or habitat, an agent of change, and threatens native biological diversity. These invasive are widely distributed in all kinds of ecosystems throughout the world, and include all categories of living organisms. Nevertheless, plants, mammals and insects comprise the most common types of invasive alien species in terrestrial environments (Raghubanshi *et al.,* 2005). Exotic weeds cause loss of biodiversity including species extinctions and changes in hydrology and ecosystem function. Some alien or exotic weeds could affect ecosystem properties by bringing nutrient to the surface from deep in the soil, thus serving as "pumps" which keep high levels of essential nutrients in circulation.

Many agriculturists have recognized that weeds despite their nuisance value do at times serve some useful purpose. Weeds often provide a protective cover against surface washing and run off. Moreover weeds are frequently used in the form of mulch around cultivated plants. Some antibiotics, as well as bio pesticides have been extracted from weeds. Apart from this many weeds are used as high poison, green manures and pollution indicators.

Weeds are comprised of the more aggressive, troublesome and undesirable elements of the World's vegetation. More than 80% of the developing world continues to rely on traditional medicines predominantly plants, for primary healthcare. The global demand for herbal medicine is not only large, but also growing. The market for *Ayurvedic* medicine is estimated to be expanding at 20% annually in India (Jeeva *et al.,* 2006).

2. METHODOLOGY

2.1. Study area

The study area Vijayamangalam village located in Perundurai taluk under Erode district. The major occupation of the peoples is agriculture and Hand loom weaving. Erode experiences hot and dry weather throughout the year. The temperature ranges from a maximum of 96°F (36°C) to a minimum of 80°F (27°C)

Field trips were made during the study period **2012- 2013.** The entire area was covered at different seasons. Specimens collected from the study area were identified carefully using the Flora of the Presidency of Madras by **J.S. Gamble** & **C.E.C. Fischer** (1915- 1935), Flora of TamilNadu Carnatic by K.M.Mathew, (1983). Identity of the plants was confirmed after critical studies with reference to authentic materials available in the Herbarium of The Botanical Survey of India, Southern circle, Coimbatore (MH) and Herbarium of Kongunadu Arts and Science College, (KASCH).

3. RESULTS

The weeds, which are best known and most highly regarded in traditional medicine, are enumerated with botanical name, local name (in Tamil), family, medicinal uses and nativity. 50 medicinal weed species belonging to 18 families and 40 genera have been recorded (Table-1). Amaranthaceae was the dominant family with 12 species, followed by Asteraceae (7 species), Malvaceae, Caesalpinaceae, Asclepiadaceae, Convolvulaceae and Nyctanginaceae (3 species each). 5 families were represented by 2 species and 6 families represented by single species. Most of the medicinal plants are common are growing in wild condition as weeds.

4. DISCUSSION

Alien species are non-native or exotic organisms that occur outside their natural adapted ranges and dispersal potential (McGeoch *et al.,* 2010). Many alien species support our farming and forestry systems in a big way. However, some of the alien species become invasive when they are introduced deliberately or unintentionally outside their natural habitats into new areas where they express the capability to establish, invade and out-compete native species. International Union for Conservation of Nature and Natural Resources (IUCN) defines Alien Invasive Species as an alien species which becomes established in natural or semi-natural ecosystems or habitat, an agent of change, and threatens native biological diversity. These invasive are widely distributed in all kinds of ecosystems throughout the world, and include all categories of living organisms. Nevertheless, plants, mammals and insects comprise the most common types of invasive alien species in terrestrial environments (Raghubanshi *et al.,* 2005).

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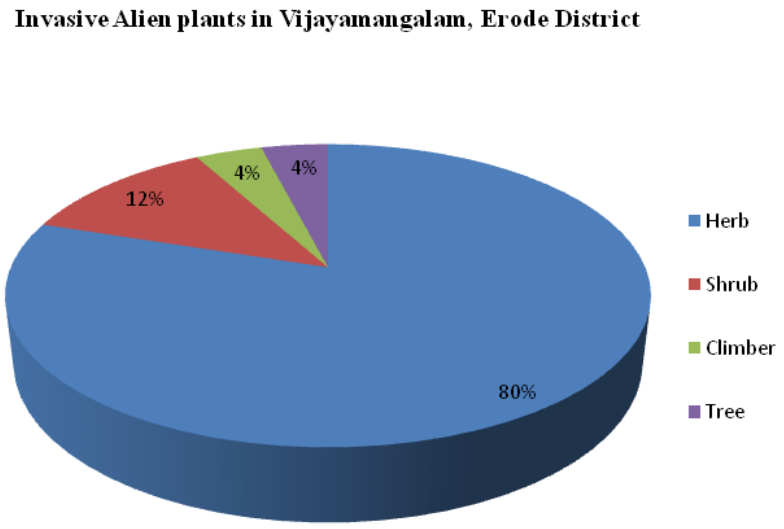

Table 1. Ethnomedicinal uses of Invasive Alien plants in Vijayamangalam, Erode District.

S.No	Botanical name	Family	Habit	Medicinal uses	Native
1.	*Argemone Mexicana*	Papaveraceae	Herb	Latex of the plant is applied topically on the site of boils	Trop. Central & South America
2.	*Cleome viscosa*	Capparidaceae	Herb	Plant is used in various disorders such as diarrhoea, fever, inflammation, liver diseases, bronchitis, skin diseases, and malarial fever.	Trop.America
3.	*Gynandropsis pentaphylla*	Capparidaceae	Herb	The decoction of leaves is used in chronic fever. Leaves areapplied to prevent the pus formation of boils. The juice of the leaves is poured into ears for earache and otalgia. The seeds paste is applied externally to expel the vermin from the hairs.	Trop.America
4.	*Abutilon indicum*	Malvaceae	Shrub	Demulcent, aphrodisiaclaxative, diuretic, sedative, astringent, expectorant, tonic, anti inflammatory,anthelmintic, and analgesic.	Trop.America
5.	*Sida acuta*	Malvaceae	Herb	Flowers paste is given in boils and burns. Root paste is applied in snake bite. Leaf is given in gastric disorder and stomach pain	Central America
6.	*Malvastrum coromandelianum*	Malvaceae	Herb	Leaves used to clean wounds; also used for dysentery.	America
7.	*Tribulus terrestris*	Zygophyllaceae	Herb	Root is used as Urinary stones, infections.	Trop.America
8.	*Abrus precatories*	Fabaceae	climber	Skin related problems, Scratches from pet animals, Abdominal Pain	Indonesia
9.	*Crotaleria pallida*	Fabaceae	Shrub	To treat urinary problems. A poultice made from the roots is applied to painful swelling of joints, and an extract of the leaves is taken as a vermifuge.	Africa
10.	*Cassia tora*	Caesalpinaceae	Herb	Leaf is useful in night blindness and leaf paste is used in cuts, boils, burns and as antiseptic treatment. Seed is given in stomach pain. Root is given in fever and abnormal child growth.	Trop. South America
11.	*Cassia occidentalis*	Caesalpinaceae	Herb	Leaves used to cure Skin diseases, Antiperiodic.	Trop. South America
12.	*Cassia hirsuta*	Caesalpinaceae	Herb	The root is pasted with cumin and taken internally to treat stomach burning after a meal.	Trop. America
13.	*Acacia nilotica*	Mimosaceae	Tree	Gargle for toothache, gum disorders, toothbrush.	Trop. America
14.	*Prosopis juliflora*	Mimosaceae	Tree	To treat eye conditions, open wounds and dermatological ailments.	Trop. America
15.	*Opuntia dillenii*	Cactaceae	Herb	Anti-diabetic, anti-inflammatory, analgesic, galactogogue, hypoglycemic, antiviral and anti-oxidant.	Trop.America
16.	*Passiflora foetida*	Passifloraceae	Herb	Decoction of fruit used for asthma and biliousness. Leaves applied to the head for headaches and giddiness.	Trop. South America
17.	*Acanthospermum hispidum*	Asteraceae	Herb	The crushed herb is used in the form of a paste to treat skin ailments and the leaf juice is reportedly used to relieve fevers.	Brazil

18.	*Ageratum conyzoides*	Asteraceae	Herb	The leaves are used in leprosy and uterine disorder, also used in killing the hairs lice. Leaf paste is applied on cuts, wounds, and burns. Leaf juice is useful skin disease and scabies disease.	Trop. America
19.	*Ageratum houstonianum*	Asteraceae	Herb	Plant juice is used externally to treat cuts and wounds.	Trop. America
20.	*Xanthium strumarium*	Asteraceae	Herb	Laxative, fattening, anthelmintic, alexiteric, tonic, digestive, antipyretic, and improves appetite, voice, complexion, and memory.	Trop. America
21.	*Parthenium hysterophorus*	Asteraceae	Herb	Flowers are useful in nasal block in cold. Leaves mixed with two or three pieces of garlics and made into fine paste, the paste is squeezed juice put in ear to stop pus flow.	Trop. North America
22.	*Tridax procumbens*	Asteraceae	Herb	Paste of leaf is given in boils, cuts & wounds. Leaf is also useful in diarrhea, dysentery and leprosy	Trop. Central America
23.	*Lagascea mollis*	Asteraceae	Herb	Leaf paste is given in cuts and wounds. Flowers are given for ear complaints.	Trop. Central America
24.	*Catharanthus pusillus*	Apocynaceae	Herb	The whole plant is used to cure cancer and diabetic	Trop.America
25.	*Daemeia extensa*	Asclepiadaceae	Climber	Leaves can be treat diarrhea among children, intestinal worms.	Trop. America
26.	*Calotropis procera*	Asclepiadaceae	Shrub	Root is used Eczema, leprosy, elephantiasis, asthma, cough and rheumatism, To treat common diseases such as fever, rheumatism, indigestion, cold, eczema and diarrhea.	Trop. Africa
27.	*Calotropis gigantean*	Asclepiadaceae	Shrub	Latex - Arthritis. Roots - Uterine disorders.	Trop. Africa
28.	*Ipomea obscura*	Convolvulaceae	Herb	The fresh plant extract is mixed with gingely oil and is used to cure cold, asthma and dry cough.	Trop. Africa
29.	*Merremia aegyptia*	Convolvulaceae	Herb	Leaves are ground and 30 ml of the extracted juice is taken once a day till cure jaundice.	Trop. America
30.	*Cuscuta chinensis*	Convolvulaceae	Herb	The paste of plant is applied on chronic ulcer, wounds. Poultice is applied on painful inflammations.	Mediterranean
31.	*Datura metal*	Solanaceae	Shrub	The fresh leaves are boiled with gingelly oil and applied topically on joints to cure swellings	T.America
32.	*Martynia annua*	Pedaliaceae	Herb	Leaves used antiepileptic and antiseptic, itching and skin affections	T.America
33.	*Pedalium murex*	Pedaliaceae	Herb	puerperal diseases, digestive tonics, ulcers, fevers, wounds, other ailments and general debility	Trop. America

34.	*Lantana camera*	Verbenaceae	Shrub	Leaves are used to treat cuts, rheumatisms, ulcers, catarrhal infection, tetanus, rheumatism, malaria, cancer, chicken pox.	Trop. America
35.	*Lippia nodiflora*	Verbenaceae	Herb	Whole plant is used for hepatitis and against abscess. Leaves are used against anti dote for snake sting .	
36.	*Boerhaavia diffusa*	Nyctanginaceae	Herb	Whole plant is used Jaundice, eye complaint, child birth and liver complaint	Trop. America
37.	*Boerhaavia verticillata*	Nyctanginaceae	Herb	Root pieces are kept in the mouse to cure mouth ulcers.	Trop. America
38.	*Mirabilis jalapa*	Nyctanginaceae	Herb	The leaves are used to reduce inflammation. A decoction of them (mashing and boiling) is used to treat abscesses. Leaf juice may be used to treat wounds.	Peru
39.	*Aerva lanata*	Amaranthaceae	Herb	treatment of diabetes mellitus, urinary calculi, hematesis, bronchitis, nasal bleeding, cough, scorpion stings, fractures, spermatorrhea, to clear uterus after delivery and also to prevent lactation	Trop. America
40.	*Aerva tomentosa*	Amaranthaceae	Herb	The herb is used for diuretic and demulcent. Its decoction is used remove swellings.	Trop. America
41.	*Alternanthera paronychioides*	Amaranthaceae	Herb	Leaves and stem used for Urine complaints.	Trop.America
42.	*Alternanthera pungens*	Amaranthaceae	Herb	Leaf decoction used for Diuretic, decoction in gonorrhea.	Trop. America
43.	*Alternanthera philoxeroides*	Amaranthaceae	Herb	Whole plant used for Allelopathic.	Trop. America
44.	*Amaranthus viridis*	Amaranthaceae	Herb	The paste of the root is applied on scorpion sting. It is used as a potherb for the alleviation of heat from the body. It is also supposed to be effective in kidney and gall bladder stones when used as potherb.	Trop. America
45.	*Digera muricata*	Amaranthaceae	Herb	Plant is used as potherb and reported as a laxative agent.	SW Asia
46.	*Alternanthera tenella*	Amaranthaceae	Herb	Ear problems	Trop. America
47.	*Celosia argentea*	Amaranthaceae	Herb	Seeds traditionally used for treatment of jaundice, gonorrhea, wounds and fever.	Trop. Africa
48.	*Amaranthus spinosus*	Amaranthaceae	Herb	Leaves are also used for gastroenteritis, gall bladder inflammation, absesses, arthritis and for the treatment of snakebites.	Trop. America
49	*Achyranthes aspera*	Amaranthaceae	Herb	asthma, bleeding, in facilitating delivery, boils, bronchitis, cold, cough, colic, debility, dropsy, dog bite, dysentery, ear complications, headache, leucoderma, pneumonia, renal complications, scorpion bite, snake bite and skin diseases	Trop. America
50.	*Achyranthes bidentata*	Amaranthaceae	Herb	Stimulate menstruation, ease menstrual pain,relieve lower back pain, canker sores, toothache, bleeding gums, nosebleeds.	Trop. America

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ANTI-HYPER LIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *SMILAX WIGHTII A.DC.* IN STREPTOZOTOCIN INDUCED MALE WISTAR ALBINO RATS

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ABSTRACT

Smilax wightii, an endemic medicinal plant is found in the shola forests at high altitudes in Nilgiri Biosphere Reserve, the Western Ghats, Southern India . The present study, was undertaken to find out the effect of methanolic extract of this plant on the body weight, fasting blood glucose levels and lipid profiles in all the streptozotocin (STZ) induced rats. The extract exerted a significant ($P<0.05$) effect in the body weight of the experimental animals when compared to the control group. Treatment with the extract and glibenclamide resulted in a significant ($P<0.01$) reduction in the fasting blood glucose levels in diabetic rats when compared to the normal. The lipid profile such as TC, TG, LDL, and VLDL contents in the serum registered a significant ($P<0.01$) hike and a decline in the HDL contents in diabetic control group, which were retrieved to near normalcy in the plant extract treated groups. The effect produced by this plant extract was comparable with that of glibenclamide. The decreased fasting blood glucose levels and lipid lowering properties clearly showed the anti-hyperlipidemic effect of *S.wightii*.

Key words: *Smilax wightii,* anti-hyperlipidemic, streptozotocin, glibenclamide

1. INTRODUCTION

Traditional medicinal herbs have served as a potential alternative source of medicine and different healthcare systems (Kamboj, 2000). Diabetes mellitus is a metabolic disorder, characterized by hyperglycemia together with impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins (Scheen, 1997). In modern medicine, no satisfactory effective therapy is still available to cure the diabetes mellitus (Piedrola *et al.*, 2001). Herbal treatments are becoming popular as the herbal preparations have no or least side effects and also they are of relatively low cost (Rajasekaran *et al*., 2001).

The genus *Smilax* has more than 300 species, found on temperature zones, tropic and subtropics worldwide and belongs to Smilacaceae (Fnaec, 2000). The roots of Smilax *wightii* have been reported to cure dysentery, amoebiasis, veneral diseases, urinary complaints, fever, spermatorrhoea, antifertility, anaemia, rheumatic-arthritis, veterinary amoebiasis and gastric complaints (Adhikari *et al*., 2010). The species of *Smilax* have been reported to contain several phytoconstituents such as dioscin, steroids smilagenin and sarsapogenin (Coimbatore BSI, 1989). Several species of Smilax are used in Chinese traditional system of medicines as anticancer, anti-inflammatory and analgesic agents (Ozoy *et al*., 2008). Therefore, the present study was aimed to assess the anti-hyperlipidemic effects of methanolic extract of *Smilax wightii* in animal models.

2. MATERIALS AND METHODS

2.1 Plant material

The plant materials were collected from Kodanadu, the Nilgiri Hills, Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist.

2.2 Preperation of extract

The whole plant materials were dried in shade after washing with cold water and then powdered using pulveriser and passed through sieve. About 100 g of dried plant powder was extracted with petroleum ether using soxhlet apparatus for 18 hours. The petroleum ether was evaporated from the extract and then the residue was re-extracted with methanol. This extract after evaporation of methanol, the filtered residue was stored at 4^0C in refrigerator for further use.

2.3 Experimental animals

Male Wistar Albino rats weighing180-250 g were obtained from Agricultural University, Animal house lab, Trissur, Kerala. The animals were fed on a standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. They were maintained in a controlled environment (12 h/12 h light/dark) and temperature (25 ± 2°C). The animals were acclimatized to the laboratory conditions for one week before starting the experiment. All the

procedures performed on animals were approved and conducted in accordance with the Institution of Animal Ethics committee and by the Regulatory body of the government (659/02/a/CPCSEA).

2.4 Toxicity studies

The acute toxicity studies were performed in adult male albino rats weighing180-250g. The animals were fasted overnight and 100- 1000 mg/kg of the test extract was provided to various groups containing six animals in each group. The treated animals were monitored for 14 days, for behaviour, general health and mortality.

2.5 Induction of diabetes

Diabetes was induced in overnight fasted Wistar albino rats by intraperitoneal (i.p injection of streptozotocin at a dose of 55 mg/kg b.wt. Forty eight hours after streptozotocin administration, blood samples were drawn from tail and glucose levels were determined to confirm diabetes. The rats with blood glucose level above 200mg/kg were considered diabetic and used for the study.

2.6 Experimental design

The rats were divided into six groups comprising of six animals in each group as follows:

Group I. : - Rats given normal saline daily for 14 days, orally (by using an intragastric catheter tube (IGC)**.** (Normal control)

Group II:- Diabetic rats given normal saline daily for 14 days , orally by using IGC. (Diabetic control)

Group III: - Diabetic rats given methanolic extract of *Smilax wightii* (MESW) at the dose of 100 mg/ Kg b.wt. daily for 14 days , orally by IGC

.**Group IV: -** Diabetic rats given methanolic extract of *Smilax wightii* (MESW) at the dose of 200 mg/ Kg b.wt. for 14 days , orally by IGC

Group V: - Diabetic rats given methanolic extract of *Smilax wightii* (MESW) at the dose of 400 mg/ Kg b.wt. for 14 days , orally by IGC

Group VI : - Diabetic rats given Glibenclamide at the dose of 600 µg/kg/b.wt. daily for 14 days , orally by IGC .

2.7 Determination of body weight

The rats from all the groups were weighed prior to the experiment and their initial body weights were recorded. The final body weights of the rats were recorded on the last day of experiment.

2.8 Estimation of fasting blood glucose (FBG) levels

Fasting blood glucose levels were measured on 0, 7 and 14 days of treatment of methanolic extract of *Smilax wightii* (MESW) supplement from the animals of all the groups. Blood samples were obtained by nicking the tails with a sharp razor and glucose concentrations were determined using a one-touch glucometer (Johnson& Johnson medical Ltd., Mumbai). The glucose concentration was read and documented from the glucometer readings. The results were expressed in terms of (mg/dl) of blood.

2.9 Estimation of lipid profile

The experimental animals from all the groups were sacrificed under light ether anaesthesia at the end of the experimental period. The rats were sacrificed by decapitation and blood was collected with anti-coagulant and the serum was used to for the estimation of lipid profile-TC-Total cholesterol (Parekh and Jung,197), TG-Triglycerides (Rice, 1970), HDL-High Density Lipoprotein (Warnick *et al*., 1985), LDL- Low Density Lipoprotein and VLDL - Very Low Density Lipoprotein (Friedwald *et al*., 1972).

2.10 Statistical Analysis

All biochemical data are expressed as Mean ± SEM. Statistical analysis was performed using one-way ANOVA using SPSS statistical analysis programme. In all cases, p-value less than 0.05 was considered to be significant.

3. RESULTS

3.1 Toxicity studies

Acute toxicity studies revealed that the methanolic extract of *Smilax wightii* (MESW) was found safe to up to a dose of 1000 mg/kg body weight. No sign of toxicity was noticed on the general health of the animals, and no death was observed in the animals when exposed to the extract.

The activity of methanolic extract of *Smilax wightii* (MESW) on body weight in streptozotocin induced diabetic rats are indicated in Table 1. There was a significant weight loss in the final body weight in the diabetic control group (Group II). A significant improvement in the body weight was observed in the extract and glibenclamide treated groups when compared to that of the diabetic control group.

Table 1: Effect of methanolic extract of *S.wightii* on the body weight of normal, diabetic induced and drug treated rats.

Treatment Groups (n=6)	Initial body weight (g)	Final body weight (g)	Body weight Gain (G↑) / loss(L↓) (g)
Group II (Diabetic control)	208.16±6.13	192.13±5.84*	16.06↓
Group III (MESW 100mg/kg)	202.84±7.84	212.17±4.36*	9.33 ↑
Group IV(MESW 200mg/kg)	198.28±3.84	211.84±4.15*	13.56↑
Group V(MESW 400mg/kg)	210.16±7.86	218.91±7.24	8.72↑
Group VI (Glibenclamide 600µg/kg)	206.82±5.91	215.05±5.16	8.23↑

Each Value is SEM of 6 animals *P <0.05 ; **P< 0.01 comparison with Normal control vs diabetic and drug treated. a , Comparison made between diabetic control to drug treated groups Level of significance a: P<0.05 , aa: P<0.01

Table 2: Effect of methanolic extract of *S. wightii* on fasting blood glucose levels of normal, diabetic induced and drug treated rats.

Treatment Groups (n=6)	Fasting Blood glucose levels (mg/dl)		
	0- day	7th day	14th day
Group I	78.13±2.41	82.18±1.93	73.93±3.54
Group II	216.13±9.13**	226.31±8.16**	229.75±9.15**
Group III	193.16±5.84**	131.28±7.24*a	102.16±4.38a
Group IV	204.83±4.33**	118.27±3.28a	93.83±2.84aa
Group V	212.48±5.83***	104.38±2.84aa	85.16±2.13aa
Group VI	218.93±6.27***	112.18±3.16aa	76.83±1.84aa

Each Value is SEM of 6 animals *P <0.05 ; **P< 0.01 comparison with Normal control vs diabetic and drug treated. a ,Comparison made between diabetic control to drug treated groups Level of significance a: P<0.05 , aa: P<0.01

Table 3: Effect of methanolic extract of *S. wightii* on the serum lipid profile of normal, diabetic induced and drug treated rats.

Treatment Groups (n=6)	TC (mg/dl)	TG(mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group I	126.84±4.16	113.88±2.14	43.18±1.84	60.89±2.12	22.77±1.02
Group II	198.15±3.16**	165.65±3.18**	23.15±1.28**	141.87±2.67**	33.13±1.33*
Group III	173.89±2.88*	141.80±3.67*	35.18±1.16*	110.03±3.62*	28.36±1.87
Group IV	156.93±2.13*	126.67±1.93 a	39.26±1.93	92.34±1.89	25.33±1.56
Group V	134.16±2.14a	118.31±1.88aa	43.91±1.88a	61.59±2.02a	23.66±1.45a
Group VI	126.48±2.09a	135.16±2.16a	41.93±1.65	57.52±1.34	27.03±1.63

Each Value is SEM of 6 animals *P <0.05 ; **P< 0.01 comparison with Normal control vs diabetic and drug treated. a ,Comparison made between diabetic control to drug treated groups Level of significance a: P<0.05 , aa: P<0.

The fasting blood glucose levels of normal, diabetic and the extract treated diabetic rats are summarized in Table 2. There was a significant (*P*<0.01) elevation in FBG level in diabetic control group when compared to normal control group. The administration of the plant extract in STZ induced diabetic rats at doses of 100,200 and 400 mg/kg b.wt produced significant (*P*<0.01) reduction in the

fasting blood glucose levels. The reducing effect of the plant extract at a dose of 400 mg/kg b.wt was found to be comparable to that of the reference drug Glibenclamide (600 mg/kg b.wt). The initial reduction in blood glucose was observed on the 7th day after the administration of the *Smilax wightii* extract. Meanwhile, on the 14th day, almost the FBG levels were reduced significantly at the dose of 400mg/kg b.wt in the extract and glibenclamide treated groups.

Table 3 depicts the changes in the levels of TC (Total Cholesterol), TG (Triglycerides), HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein) and VLDL (Very Low Density Lipoprotein) of the experimental animals. Diabetes induced by STZ in rats significantly (P<0.01) elevated the TC, TG, LDL, VLDL levels and decreased the HDL levels in diabetic control compared with normal control rats. In the present study, administration of MESW at dose of 100, 200 and 400 mg/kgb.wt to the diabetic rats showed significant (P<0.05, P<0.01) reduction in TC, TG, LDL, and VLDL levels and increase in HDL levels than diabetic control rats.

4. DISCUSSION

Diabetes is a major health problem affecting major populations worldwide. It is a chronic disorder in metabolism of carbohydrates, proteins, and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance. There are more than 30 million people with diabetes mellitus in India and the incidence is increasing. Decreased physical activity, increasing obesity, stress and changes in food consumption have lead to the cause of diabetes (Jarald *et al*., 2008).

The present study was aimed to investigate the anti-hyperlipidemic activity of methanoic extract of *S. wightii* (MESW) in STZ-induced diabetic rats. The results of the study revealed that MESW at the doses of 400 mg/kg b.wt. significantly normalized elevated blood glucose level and restored serum lipid profiles towards normal values.

Streptozotocin STZ, a highly cytotoxic agent of pancreatic β-cells induces diabetes by damaging the cells and causes reduction in insulin secretion (Esmaeli and Yazdanparas, 2004). The increased glucose level in the diabetic control animals may be due to the destruction of the pancreatic cells caused by the STZ induction. The increased levels of plasma glucose in STZ-induced diabetic rats were lowered by the administration of *Smilax wightii* extract. Diabetes affects both glucose and lipid metabolism (Sperling and Saunders, 2000).

Hyperlipidemia was reported as common in adults with diabetes and it is characterized most often by increased triglyceride and reduced HDL cholesterol levels. This is generally observed in both type 1 and type 2 diabetes, representing the defect of insulin action in each, either due to inadequate secretion or resistance (Garg and Grundy, 1990). Increased fatty acid concentrations also increased the β-oxidation of fatty acids, producing more acetyl-CoA and cholesterol in diabetics (Yokogoshi and Oda, 2000, Mard *et al*., 2010). Alterations in plasma lipoprotein metabolism are common in diabetes, which tend to exaggerate any pre-existing tendencies towards elevated lipid levels (Merzouk *et al*., 2004). The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atherosclerosis (Bopanna *et al*., 1997). Previous studies in *Smilax chinensis* has reported similar results in the fasting blood glucose levels and lipid profile in Streptozotocin induced diabetic rats (Venkidesh *et al*., 2010). On the basis of the present study, it could be concluded that the methanolic extract of *S.wightii* exerted a significant antidiabetic and antihyperlipidemic effect. This could be due to different types of active principles present in the extract which serve as a good adjuvant in the present armamentarium of antidiabetic drug and has therapeutic value.

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