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THE NEED OF SPIRITUAL ENDEAVOURS FOR AN INTELLECTUAL EXISTENCE: A RE-READING OF HERMANN HESSE'S *SIDDHARTHA*

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ABSTRACT

HermannHesse's*Siddhartha* is focussed on the tribulations of Siddhartha through his quest for inner peace. His journey begins as a young Brahmin who yearned to unwind the complexities of his existence. He ends as an old sage who has found peace within himself and his surroundings. Through this novel Hesse, allows the readers to trace the maturation process both through his experiences and people with whom he comes to contact.Born of Protestant family in Germany he was tremendously influenced by ancient Hindu Philosophy. His search for Eastern mysticism can be seen in this novel. He could recognise the unity of vision in Hinduism and Buddhism. He could detect the essence of Indian spiritual life led by many people.Siddhartha, the protagonist of the novel, identify the path for attaining enlightenment and he goes in the way where he experiences the worldly life hurdles. He could understand that the enlightenment does not come from mastering either the material or spiritual world but from finding the common ground between these polarities of existence. Siddhartha finds enlightenment only when he understandsOm, the unity of polarities.

Keywords: Spiritual endeavours, Hermann Hesse's Siddhartha.

In an atmosphere of collective identity, ethical obligation and political responsibility by narrowing the diverse geographical spaces,a re-reading of Hermann Hesse's fiction becomes significant. My paper intends to examine the philosophical implications in Hesse's *Siddhartha* which have inspired to relocate the divine in us. The phenomenon of globalisation has destabilised the prevailing concepts of cultural diversity and in such juncture the main concern is the constructive and meaningful efforts to resist spiritual degeneration .

Siddhartha is focussed on the tribulations of Siddhartha through his quest for inner peace. His journey begins as a young Brahmin who yearned to unwind the complexities of his existence. He ends as an old sage who has found peace within himself and his surroundings. Through this novel Hesse allows the readers to trace the maturation process both through his experiences and people with whom he comes to contact.

Hermann Hesse was born of Protestant family in Germany. A democrat in politics and an individualist in outlook, he confessed that he was tremendously influenced by ancient Hindu Philosophy. He was one of the most cultured and sensitive of contemporary German writers .His western upbringing and the conscious application of the intellect helped Hesse in discerning the spiritual greatness of Buddhism and Hinduism. He was much fascinated by the endless patience and kindness and this essence of experience, Hesse puts across to the western readers through the character of Siddhartha. He is a bundle of contradictions till he realises the multiplicity in appearances and the timelessness in this temporal life. Hesse became a quester, a seeker after truth. As every seeker he finally sees a unity in diversity. His search for truth also confirmed this strong conviction that mankind has a meaning. It must be remembered that without understanding the religious consciousness of India a novel like *Siddhartha* could not have been written. His friend and servant, Govinda, the ferry man, Kamala; the prostitute, Vasudeva , Kamaswamy; the merchant-all of them from various socio-structural patterns taught him the meaning of life. Even the river in the novel had something special to tell him. It was always the same yet every moment it was new. The river becomes to him the symbol of universal beauty, harmony and love.

Hermann Hesse the author of *Siddhartha* was very much influenced by the hidden spirit of India. Buddha's influence and and his search for religious serenity in Eastern mysticism can be seen in *Siddhartha*. Hesse's parents and grandparents had long connections with India. His grandfather came to India in 1836.Marie Hesse, his mother was born at Thalassery, in North Malabar. Gundert wrote the first Malayalam –English dictionary, the first Malayalam grammar book and had translated the Bible in to

Malayalam. In Gundert's family Hinduism, Buddhism, Confucianism, and Protestantism, mingled without contradictions and this influenced the young mind of Hesse. He could recognize the unity of vision which is the essence of Indian life. His western upbringing and the conscious application of the intellect helped Hesse in discerning the spiritual greatness of Hinduism and Buddhism.

The story begins by painting a picture of Siddhartha as a perfect son: smart, athletic, obedient, and handsome. However, he eventually sees the limitations of the Brahmin life, and leaves his home to join the ascetics with his companion Govinda. The two set out in the search of enlightenment. After seeing the limitation of asceticism, the two journey to meet the Buddha. Govinda is immediately impressed and takes refuge in the Buddha. Siddhartha respects the Buddha's enlightenment, but realizes that no teaching, not even the Buddha's, can capture enlightenment.

The second half of the book starts with Siddhartha impressing a beautiful, wealthy courtesan. She sets him up with a job so that he can afford the beautiful things that will impress her. Initially seeing this as a game for children, he eventually finds himself caught up in the trading, drinking, and gambling of a merchant life. "Then Siddhartha knew the game was finished, that he could play it no longer. A shudder passed through his body" (*Siddhartha*,67). He leaves again for the forest. After settling into a nice life sharing ferryman duties with a wise friend, Siddhartha finds out him fathered a son. He attempts to raise the boy in this simple life, but the boy gets frustrated and returns to the city. Siddhartha finally feels the sorrow of love, which leads to a deep compassion for all of his fellow humans. With Vasudeva , his guide Siddhartha learns to listen the river and hear the holy 'Om'.

> …all the voices ,all the yearnings, all the sorrows, all the pleasures….all of them together was the stream of events ,the music of life.(*Siddhartha*,107)

Siddhartha studies the river for many years, and Vasudeva teaches Siddhartha how to learn the many secrets the river has to tell. In contemplating the river, Siddhartha has a revelation: Just as the water of the river flows into the ocean and is returned by rain, all forms of life are interconnected in a cycle without beginning or end. Birth and death are all part of a timeless unity. Life and death, joy and sorrow, good and evil are all parts of the whole and are necessary to understand the meaning of life. By the time Siddhartha has learned all the river's lessons, Vasudeva announces that he is through with his life at the river. He retires into the forest, leaving Siddhartha to be the ferryman. He asserts his own belief in time and unity that brings him much closer to inner peace. He hears the universal within the Om and he understood that only love can bring peace to the world.

Experience is the aggregate of conscious events experienced by a human in life – it connotes participation, learning and knowledge. In Hesse's novel *Siddhartha*, experience is shown as the best way to approach understanding of reality to attain enlightenment. Hesse's crafting of Siddhartha's journey shows that understanding is attained not through scholastic, mind-dependent methods, nor through immersing oneself in asceticism and the carnal pleasures of the world. While these individual events only bring about more samsara, they cannot be considered distractions because it is the totality of these experiences that allow Siddhartha to attain understanding.

In *Siddhartha*, Siddhartha finds that enlightenment does not come from mastering either the material or spiritual world but from finding the common ground between these polarities of existence. In the first third of the book, Siddhartha rejects the material world. The Brahmins, Samanas, and Buddhists all maintain that the material world is illusion, or Maya, that distracts a seeker from the spiritual truth. Adopting this belief, Siddhartha completely denies his body and, instead, focuses his efforts on refining his mind and memorizing the knowledge his teachers pass along to him. In the second third of the book, Siddhartha rejects the spiritual world and enters the material world, but relentlessly pursuing carnal desire does not lead him to wisdom either. Siddhartha battles with other polar opposites as well, such as time/timelessness and attachment/detachment, but in these, too, he finds that embracing one and rejecting the other does not lead to enlightenment. The river suggests this battle visually: the opposing banks represent the polarities, and the river itself represents the ideal union of them. Siddhartha finds enlightenment only when he understands Om, the unity of polarities. He achieves transcendence when he can accept that all is false and true at the same time, that all is living and dead at the same moment, and that all possibilities are united in the spirit of the universe

Siddhartha is often considered the high point of Hesse's art in fiction, as well as the pinnacle of his fascination with orientalism. The novella is concerned with the individual's search for truth and identity by means of what Hesse termed the inward journey, a recurring theme throughout his works.

During that period, American youth, involved in an era of cultural upheaval, identified with the title character and his struggle to transcend meaninglessness and materialism through mysticism and love.

Hesse's *Siddhartha* reflects much of the literary and intellectual history of Germany and Western Europe during the first decades of the twentieth century. In particular, the work has many points in common with the Romantic Movement, neo-romanticism, and expressionism. The importance of what Hesse termed the individual's struggle to transcend the materialism of bourgeois society through art, mysticism, and love—is especially palpable in *Siddhartha.* Highly influenced by the philosophy of Friedrich Nietzsche, Hesse had vowed to reject traditional religion and morality and lead a life of individualism and isolation. Siddhartha also rejects traditional religion and morality, and ultimately finds that pure individualism is an embrace of unity, with love as the synthesizing agent. Thus, *Siddhartha* fits well both in the genres of the novel of education, and the *Bildungsroman.* Hesse addressed in *Siddhartha,* as in most of his other works, characters who struggle to come to terms with themselves, individuals who passionately attempt self-realization.

Hesse's literary creations are fictional attempts to counter vacuum born out of the cultural diversities of the world. *Siddhartha* is the sum total of ancient wisdom, Upanishadic wisdom and the very essence of the experience of the Buddha. It is the journey through the self, till one discovers the true place of one's self in this world of diversity and illusion. The author's ability to universalize private agony and personal crises, as demonstrated in *Siddhartha,* has allowed Hesse to achieve an on-going international popularity. *Siddhartha* is a colourful novel about an Indian youth's long and spiritual quest for the answer to the enigma of man's role on earth.

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FROM SUBSERVIENCE TO SELF-ASSERTION: TRANSFORMING FEMALE IDENTITY IN AMY TAN'S THE KITCHEN GOD'S WIFE

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ABSTRACT

Amy Tan, the well-known Chinese American author has won acclaim for her depiction of various aspects of the mother-daughter relationship and the depiction of the female struggle against patriarchal society. *The Kitchen God's Wife* is the story of Winnie Louie, a Chinese immigrant, as told to her American-born dentist daughter, Pearl. Weili, as Winnie is known in China, is the victim of an abusive marriage. She is the wife of the cruel Wen Fu, whom she later mentally renames "The Kitchen God" Weili endures considerable physical, mental and emotional torture with passivity because she the ideal of the subservient Chinese wife has been inculcated in her. Weili's perspective opens up when she meets Jimmy Louie, a Chinese American soldier. He gives her a new, Western name, that of "Winnie," and paves a way for her search for an identity apart from her husband. Winnie has to face a series of horrendous trials before she marries Jimmy and settles in the United States. After Jimmy's death, Winnie forges out a new, independent life for herself. She gives her daughter the courage to overcome her illness by fashioning a new goddess from a defective statuette. Winnie names this symbolic recreation of her own self as "The Kitchen God's Wife." From victim, she becomes a victor, capable of nurturing and protecting her progeny.

Keywords: Amy Tan, Kitchen god's wife.

The Kitchen God's Wife has been described as "an extremely complex postmodern literary novel" (*Endnotes.com)*. Amy Tan's book, with its strong autobiographical note depicts life in old China as well as the immigrant and second-generation Chinese American experience. It begins with the narrative of Pearl Louie Brandt, a typical second-generation Chinese American woman married to a white American. She visits her mother widowed mother, Winnie Louie, from whom she has been estranged for a number of years. During the visit, Winnie relates the story of her past in pre-Communist China. She also tells Pearl that she is not the daughter of Jimmy Louie, the gentle Baptist pastor who passed away when Pearl was a teenager, but that of Winnie's first husband, a wicked man named Wen Fu. This narration creates a closer understanding between mother and daughter and also paves the way for Winnie to come to terms with herself.

Winnie, whose Chinese name is Jang Weili, is brought up in strict accordance with the ideology of Confucius which relegates women to a secondary position in society. In the words of Judith Caesar, "*The Kitchen God's Wife* acknowledges the historical role played by patriarchal ideology in its moulding of Chinese women." Women in China were trained to be gentle and submissive, with the pleasing of their husbands as their main aim in life. Women like Weili's mother, who was an educated "Shangai woman" were ostracized by society.

Weili is given in marriage to the attractive but greedy and wicked Wen Fu. Her innocence is exploited by her husband, who makes her a slave to his whims and fancies. He claims absolute mastery over her body and mind ("Character"). He proves to be dangerously jealous and cruel. He crushes Weili's self-esteem by insulting her in public, making her suffer untold miseries in private, demanding that she beg and fawn on the ground for imaginary slights. Wen Fu and his family shamelessly spend her dowry and try ways and means to take advantage of her father's wealth and position. Weili loses several children due to Wen Fu. Particularly pathetic is the death of Yiku. She becomes retarded after being hit by her father who wants to punish Weili. While the little girl lies seriously ill, Wen Fu plays cards and refuses to take her to a doctor until it is too late. After a point of time, Weili begins to abort the life in her womb to save her offspring from the unhappy life she foresees for them. It is to be noted that Wen Fu treats his concubines and his other women in an equally atrocious manner. Weili speaks of her huaband's ill-treatment: "I was not angry. I did not know I was supposed to be angry. This was China. A woman had no right to be angry" (Tan 170).

Weili's life begins to change when she meets Jimmie Louie, and American-born Chinese soldier

during the war. Jimmie gives her a new name, 'Winnie,' echoing the word 'win.' This renaming is the beginning of a new life for her. After a few years, she makes the brave decision to break away from her abusive marriage. She is heartened to do so when she learns of her cousin Peanut's escape from her mentally-retarded husband. She also visits the shelter that the mother of one of her classmates runs for women who wish to leave unbearable marriages. She is delayed by the legal action that Wen Fu takes against her. It is the patriarchal system that enables Wen Fu to exploit powerful institutions such as the law and media to represent himself as having lost his son, wife and property. Winnie is prepared to go to jail rather than continue as the subservient wife of a heartless man. In prison, she is considered a heroine by some and by others, an immoral and heartless woman who lets her son die in order to enjoy the love of a foreigner. However, Winnie retains the true devotion of the pure-hearted Jimmie Louie, who arranges for her emigration to the United States as his wife.

After her release, Winnie is determined to legalise her marriage to Jimmie by getting a proper divorce from Wen Fu. Even though her tickets are ready and she is has been acknowledged as the wife of Jimmie in the United States, her stubborn nature refuses to settle for nothing less than the truth. She formulates a plan with the help of her friend Helen and Wan Betty the telegraph operator to publicly force Wen Fu to retract his lies about not divorcing her previously. She makes him sign another divorce paper. However, Wen Fu has his revenge. He gains entry into her home, rapes her at gunpoint and makes her beg him for her air tickets. Winnie manages to get hold of the gun and would have shot him but for the timely intervention of Helen. She escapes to the United States, but only with Wen Fu's child in her womb. Winnie is able to begin her life again in spite of the trauma she faces. She brings up her daughter Pearl and the son she has later. After Jimmie's early death, she leads an independent life. This continues even in her old age. Things come to a head when Pearl reveals to her that she is suffering from multiple sclerosis, an incurable condition.

The title of the novel is based on the legend of the Kitchen God. In Chinese folklore, he was a man named Zhang who was married to a wonderful lady named Guo, who had all the virtues expected of a typical traditional Chinese wife. Zhang lacked nothing in life, but suffered from the malaise of discontent. He acquires a mistress called Lady Li. Zhang starts ill-treating his wife and finally throws her out of his house. As fate would have it, Lady Li proves faithless and leaves him for another man. Zhang is reduced to the condition of a beggar. A kindly woman offers him food and shelter. To his shock and dismay, Zhang finds one day that his benefactress is his own wife. Unable to face what seems to his ego a disgrace, he commits suicide by throwing himself into the fireplace in the kitchen. In a rather puzzling manner, he is elevated to the status of the Kitchen God with the power to tell the Jade Emperor of heaven who deserved reward and who punishments. The fate of his guiltless wife Guo is obscure, no particular reward coming to her share for her charitable and forgiving nature. This reflects the injustices in the traditional Chinese society, where women are expected to be subservient at all times to the male ego.

The Kitchen God is supposed to be a capricious dictator, one who expects to be pleased and served unquestioningly, and whose patriarchal superiority is unshakeable. He reminds Winnie of Wen Fu and the way he expected her to grovel before him in order to appease uncontrollable anger. "Winnie does not feel that the Kitchen God is worth honoring" ("Mythology"). She feels that "His wife is the good one not him" (Tan 12).Winnie decides that such a cruel god with his whims and fancies would not bring her daughter the kind of luck she needed. Therefore, she throws the picture of Zhang into the fire, setting off the fire alarm in the process. This incongruous intrusion of the modern, Western sound at this conjecture is momentarily misinterpreted by Winnie as Wen Fu's angry cry. She soon realizes the delusion created by her deeply-ingrained fear and feels relief. She remembers that the wicked god as well as her tyrannical husband is dead.

Winnie's mind is set upon finding an appropriate statuette to take the place of the now defunct Kitchen God. She rejects several deities shown to her at the shop and instead, chooses a small statuette which has a defect. There is no name written on its pedestal. Winnie's imagination is heightened by the possibility of creating a new goddess to give her daughter the new kind of luck to see her through her new, American-style illness. She writes in gold paint the name of the new goddess-Lady Sorrowfree. This is perhaps a reflection of the name she gives her stillborn baby girl, Mouchou, which also means "sorrowfree." Winnie felt that all female children were born for grief, and that her little girl had escaped her fate. Thus, she consoled herself for the loss of her child, which she superstitiously attributed to the dropping of a pair of scissors.

When Winnie lost her child in China, she was a helpless housewife, having no control over her

body, mind or fortunes. Therefore, she was unable to offer protection to any of her children. Now, she was an American citizen, a lady making her own living, leading an independent life, well aware of her rights. As a mother, she had the freedom and the capacity to save her daughter Pearl from the fate of her children born in China. As a typical Chinese housewife, she had no right to any choice. She was not appreciated for any of her sacrifices. In a way, she was similar to Guo, the Kitchen God's wife. Feeling the need for recognition and empowerment, Winnie identifies herself with Guo and elevates the lady to the status of a deity, Lady Sorrowfree. She gives the goddess an altar, making her quite independent of her husband. It is noteworthy that the new goddess occupies the place of the Kitchen God, and is portrayed as laughing and crying out "Yes, yes, yes!"(Tan 413) in approval when he perishes in the fire. This reflects Winnie's fantasies of Wen Fu's death during his service as a pilot during the war. These wishes of Winnie are wicked but merely the feelings of a desperate animal caged and ill-treated beyond endurance, and that of a mother forced to witness the death of her children due to callous neglect.

The newly-created mother goddess is Winnie herself, who knows instinctively the kind of luck that Pearl needs. She has a deep-seated feeling that the multiple sclerosis that her daughter suffers from is the inheritance of her father. As the first step towards freeing her daughter from his evil influence, she has exorcised his memory by destroying the picture of the Kitchen God. As a second step, she creates a positive flow of energy by installing a new deity. The male here is identified with the negative and the female with the positive, which is an inversion of the yin-yang theory of Chinese philosophy. This in itself is an assertion of the feministic viewpoint that Winnie acquires upon her migration to the United States.

Winnie also takes a third and more tangible step in the form of a proposed journey to China to treat her daughter with the life-giving water of the spring in and consult traditional Chinese herbal doctors. She is purged of many sorrows. She had secret fears about Wen Fu getting to know about Pearl's existence, guessing that she was his daughter and coming to claim her. She is persuaded by Helen to reveal to Pearl the truth about her past. Winnie also finds that both Helen and Auntie Du knew all along that Pearl was not Jimmie Louie's child. Nevertheless, they approved of her deception. All this proves a relief to Winnie. She finally gets the support of the only family she has now. The news of Wen Fu's death, sent by Wan Betty soothes her fears. She feels that now she is Lady Sorrowfree, a re-created being. She is not one who has never known any sorrow, but one who overcomes all sorrows and emerges triumphant. Winnie comes into terms with herself, redefines herself, and becomes a divine being, a fully-realised mother.

Winnie gains the confidence that she could fight and win the battle of illness on behalf of her daughter. The effort, after all, is similar to her heroic struggle to escape from Wen Fu. Thus, from the frail lady Jang Weili emerges the formidable matriarch Winnie Louie, filled with the wisdom to lead, the ability to bless with luck and the foresight and fortitude to change adversity into good fortune. As Adams puts it, Lady Sorrowfree has "endured all, received no credit for the work she has done, and is still strong

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ON $\alpha\delta$-REGULAR SPACES

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ABSTRACT

The concept of $\alpha\delta$-closed sets was introduced by Devi *et al.,* 2012. The aim of this paper is to consider and characterize $\alpha\delta$-regular spaces and study some of their properties.

Keywords: αδ-regular spaces.

1. INTRODUCTION

The importance of general topological spaces rapidly increases in many fields of applications such as data mining. Information systems are basic tools for producing knowledge from data in any real-life field. Topological structures on the collection of data are suitable mathematical models for mathematizing not only quantitative data but also qualitative ones.

Generalized open sets play a very important role in General Topology and they are now the research topics of many topologists worldwide. Indeed a significant theme in General Topology and Real analysis concerns the variously modified forms of continuity, separation axioms etc. by utilizing generalized open sets. One of the most well known notions and also an inspiration source is the notion of $\alpha\delta$-open (Devi *et al.,* 2012) sets introduced by R.Devi, V.Kokilavani and P.Basker. In this paper, we will continue the study of related functions with $\alpha\delta$-open and $\alpha\delta$-closed sets. We introduce and characterize the concept of $\alpha\delta$-regular spaces and study some of their properties.

2. PRELIMINARIES

Throughout the present paper, spaces X and Y always mean topological spaces. Let X be a topological space and A a subset of X. The closure of A and the interior of $A$are denoted by $Cl(A)$ and I$nt(A)$, respectively. A subset A is said to be regular open (resp. regular closed) if $A = int(cl(A))$ (resp. $A = cl(int(A))$, The δ-interior (Velico, 1968) of a subset A of X is the union of all regular open sets of X contained in A and is denoted by $Int_\delta(A)$.The subset A is called δ-open (Velico, 1968) if $A = Int_\delta(A)$, i.e., a set is δ-open if it is the union of regular open sets. The complement of a δ-open set is called δ -closed. Alternatively,a set $A \subset (X, \tau)$ is called δ-closed (Velico, 1968) if $A = cl_\delta(A)$,where $cl_\delta(A) = \{x/x \in U \in \tau \Rightarrow int(cl(A)) \cap A \neq \varphi\}$. The family of all δ-open (resp. δ-closed) sets in X is denoted by $\delta O(X)$ (resp. $\delta C(X)$). A subset A of X is called semiopen (Noiri, 1998) (resp. α-open (Devi *et al.,* 2012), δ-semiopen[2]) if $A \subset cl(int(A))$ (resp.$A \subset int(cl(int(A)))$, $A \subset cl(Int_\delta(A)))$ and the complement of a semiopen(resp. α-open, δ-semiopen) are called semiclosed(resp. α-closed, δ-semiclosed).The intersection of all semiclosed (resp. α-closed, δ-semiclosed) sets containing A is called the semi-closure(resp. α-closure, δ-semiclosure) of A and is denoted by $scl(A)$ (resp.$\alpha cl(A)$, δ-$scl(A)$). Dually, semi-interior(resp. α-interior, δ-semi-nterior) of A is defined to be the union of all semiopen (resp. α-open,δ-semiopen) sets contained in A and is denoted by $sint(A)$ (resp.$\alpha int\,(A)$, δ-$sint(A)$). Note that δ-$scl(A) = A \cup int(cl_\delta(A))$ and δ-$sint(A) = A \cup cl(Int_\delta(A))$.

We recall the following definition used in sequel.

2.1. Definition (Devi *et al.,* 2012) A subset A of a space X is said to be

(a) An α-generalized closed (αg-closed) set if $\alpha cl(A) \subseteq U$ whenever $A \subseteq U$ and U is α-open in (X, τ).

(b) A $\alpha\delta$-closed set if $cl_\delta(A) \subseteq U$ whenever $A \subseteq U$ and U is αg-open in (X, τ).

2.2. Definition A function $f: (X, \tau) \to (Y, \sigma)$ is called

(a) $\alpha\delta$-continuous (Kokilavani and Basker, 2006) if $f^{-1}(V)$ is $\alpha\delta$-closed in (X, τ) for every closed set V of (Y, σ).

(b) $\alpha\delta$-irresolute (Kokilavani and Basker, 2006) if $f^{-1}(V)$ is $\alpha\delta$-closed in (X, τ) for every $\alpha\delta$-closed set V of (Y, σ).

(c) semi-closed (Noiri, 1998) if $f(V)$ is semiclosed in Y for every closed set V in X.

(d) pre-closed (Noiri, 1998) if $f(V)$ is closed in Y for every semi-closed set V in X.

2.3. Definition **(**Kokilavani Varadharajan and Basker Palaniswamy, 2013) A topological space (X, τ) is called

(a) $T_0^{\#\alpha\delta}$ if for any distinct pair of points in X, there is a $\alpha\delta$-*open* set containing one of the points but not the other.

(b) $T_1^{\#\alpha\delta}$ if each pair of distinct points x and y in X there exists a $\alpha\delta$-*open* set U in X such that $x \in U$ and $y \notin U$ and a $\alpha\delta$-*open* set V in X such that $y \in V$ and $x \notin V$.

(c) $T_2^{\#\alpha\delta}$ if for each pair of distinct points x and y in X there exist $\alpha\delta$-*open* sets U and V such that $U \cap V = \varphi$ and $x \in U, y \in V$.

3. $\alpha\delta$-REGULAR SPACES

In this section, we introduce and study $\alpha\delta$-regular spaces and some of their properties.

3.1. Definition A topological space X is said to be $\alpha\delta$-regular if for each closed set F and each point $x \notin F$, there exist disjoint $\alpha\delta$-open sets U and V such that $x \in U$ and $F \subset V$.

3.2. Definition A function $f: (X, \tau) \to (Y, \sigma)$ is called

(a) semi-$\alpha\delta$-continuous if $f^{-1}(V)$ is $\alpha\delta$-closed in (X, τ) for every semi-closed set V of (Y, σ).

(b) $\alpha\delta$-open if $f(V)$ is an $\alpha\delta$-open in (Y, σ) for every open set V in (X, τ).

(c) $p\alpha\delta$-open if $f(V)$ is an $\alpha\delta$-open in (Y, σ) for every semi-open set V in (X, τ).

(d) $(Q, \alpha\delta)$-open if $f(V)$ is an open in (Y, σ) for every $\alpha\delta$-open set V in (X, τ).

(e) Strongly $\alpha\delta$-open if $f(V)$ is an $\alpha\delta$-open in (Y, σ) for every $\alpha\delta$-open set V in (X, τ).

3.3. Theorem Every $\alpha\delta$-regular $T0$-space is $T_2^{\#\alpha\delta}$.

Proof. Let $x, y \in X$ such that $x \neq y$. Let X be a $T0$-space and V be an open set which contains x but not y. Then $X - V$ is a closed set containing y but not x. Now by $\alpha\delta$-regularity of X there exist disjoint $\alpha\delta$-open sets U and W such that $x \in U$ and $X - V \subset W$. Since $y \in X - V, y \in W$. Thus for $x, y \in X$ with $x \neq y$, there exist disjoint open sets U and W such that $x \in U$ and $y \in W$. Hence X is $T_2^{\#\alpha\delta}$.

3.4. Theorem In a topological space X, the following conditions are equivalent:

(a) X is $\alpha\delta$-regular.

(b) For every point $x \in X$ and open set V containing x there exists a $\alpha\delta$-open set U such that

$x \in U \subset \alpha\delta_{Cl}(U) \subset V$,

(c) For every closed set F, $F = \cap \{\alpha\delta_{Cl}(V): F \subset V \text{ and } V \text{ is } \alpha\delta - \text{open set of } X\}$,

(d) For every set A and an open set B such that $A \cap B \neq \varphi$, there exists $\alpha\delta$-open set O such that

$A \cap O \neq \varphi$ and $\alpha\delta_{Cl}(O) \subset B$,

(e) For every non empty set A and closed set B such that $A \cap B \neq \varphi$, there exist disjoint $\alpha\delta$-open

sets L and M such that $A \cap L \neq \varphi$ and $B \subset M$.

Proof. $(a) \Rightarrow (b)$: Let V be an open set containing x. Then $X - V$ is closed set not containing x. Since X is $\alpha\delta$-regular, there exist $\alpha\delta$-open sets L and U such that $x \in U, X - V \subset L$ and $U \cap L = \varphi$. This implies $U \subset X - L$. Therefore, $\alpha\delta_{Cl}(U) \subset \alpha\delta_{Cl}(X - L) = X - L$, because $X - L$ is $\alpha\delta$-closed. Hence $\in U \subset \alpha\delta_{Cl}(U) \subset X - L \subset V$. That is $\in U \subset \alpha\delta_{Cl}(U) \subset V$.

$(b) \Rightarrow (c)$: Let F be a closed set and $x \notin F$. Then $X - F$ is an open set containing x. By (b), there

is a $\alpha\delta$-open set U such that $x \in U \subset \alpha\delta_{Cl}(U) \subset X - F$. And so, $F \subset X - \alpha\delta_{Cl}(U) \subset X - U$. Consequently $X - U$ is $\alpha\delta$-closed set not containing x. Put $V = X - \alpha\delta_{Cl}(U)$. This implies $F \subset V$ and V is $\alpha\delta$-open set of X and $x \notin \alpha\delta_{Cl}(V)$, implies

$$\cap\{\alpha\delta_{Cl}(V): F \subset V \text{ and } V \text{ is } \alpha\delta - \text{open set of } X\} \subset F \qquad (1)$$

But F is closed and every closed set is $\alpha\delta$ closed. Therefore

$$F \subset \cap\{\alpha\delta_{Cl}(V): F \subset V \text{ and } V \text{ is } \alpha\delta - \text{open set of } X\} \qquad (2)$$

is always true. From (1) and (2),

$$F = \cap\{\alpha\delta_{Cl}(V): F \subset V \text{ and } V \text{ is } \alpha\delta - \text{open set of } X\}.$$

$(c) \Rightarrow (d)$: Let $A \cap B \neq \varphi$ and B is open. Let $x \in A \cap B$. Then $X - B$ is a closed set not containing x. By (c), there exists a $\alpha\delta$-open set V of X such that $X - B \subset V$ and $x \neq \alpha\delta_{Cl}(V)$. Put $O = X - \alpha\delta_{Cl}(V)$, then O is $\alpha\delta$-open set of X, $x \in A \cap O$ and $\alpha\delta_{Cl}(O) \subset \alpha\delta_{Cl}(X - V) = X - V \subset B$. Hence $\alpha\delta_{Cl}(O) \subset B$.

$(d) \Rightarrow (e)$: If $A \cap B = \varphi$, where A is non empty and B is closed, then $A \cap (X - B) \neq \varphi$ and $X - B$ is open. Therefore by (d), there exists $\alpha\delta$-open set L such that $A \cap L \neq \varphi$ and $L \subset \alpha\delta_{Cl}(L) \subset X - B$.

$(e) \Rightarrow (a)$: Let F be a closed set such that $x \notin F$, then $\{x\} \cap F = \varphi$. By (e), there exist disjoint open sets L and M such that $\{x\} \cap L \neq \varphi$ and $F \subset M$, which implies $x \in L$ and $F \subset M$. Hence, X is $\alpha\delta$-regular.

3.5. Theorem If $f : X \to Y$ is continuous bijective, $\alpha\delta$-open (resp. $p\alpha\delta$-open) function and X is a regular (resp. s-regular) space, then Y is $\alpha\delta$-regular.

Proof. Let F be a closed set in Y and $y \notin F$. Take $y = f(x)$ for some $x \in X$. Since f is continuous $f^{-1}(F)$ is closed set in X such that $x \notin f^{-1}(F)$. Now X is regular (resp. s-regular), there exist disjoint open (resp. semi-open) sets U and V such that $x \in U$ and $f^{-1}(F) \subset V$. That is, $y = f(x) \in f(U)$ and $F \subset f(V)$. Since f is $\alpha\delta$-open (resp. $p\alpha\delta$-open) function $f(U)$ and $f(V)$ are $\alpha\delta$-open sets in Y and f is bijective $f(U) \cap f(V) = f(U \cap V) = f(\varphi) = \varphi$. Therefore, Y is $\alpha\delta$-regular.

3.6. Theorem If $f : X \to Y$ is semi continuous bijective, $p\alpha\delta$-open function and X is semi regular space, then Y is $\alpha\delta$-regular.

Proof. Let F be a closed set in Y and $y \notin F$. Take $y = f(x)$ for some $x \in X$. Since f is semi continuous $f^{-1}(F)$ is semiclosed set in X and $x \notin f^{-1}(F)$. Now X is semi regular, there exist disjoint semiopen sets U and V such that $x \in U$ and $f^{-1}(F) \subset V$. That is, $y = f(x) \in f(U)$ and $F \subset f(V)$. Since f is $p\alpha\delta$-open function $f(U)$ and $f(V)$ are $\alpha\delta$-open sets in Y and f is bijective $(U) \cap f(V) = f(U \cap V) = f(\varphi) = \varphi$. Therefore, Y is $\alpha\delta$-regular.

3.7. Theorem If $f : X \to Y$ is continuous surjective, strongly $\alpha\delta$-open (resp. $(Q, \alpha\delta)$-open) function and X is $\alpha\delta$-regular space, then Y is $\alpha\delta$-regular (resp. regular).

Proof. Let F be a closed set in Y and $y \notin F$. Take $y = f(x)$ for some $x \in X$. Since f is continuous surjective $f^{-1}(F)$ is closed set in X and $x \notin f^{-1}(F)$. Now since X is $\alpha\delta$-regular, there exist disjoint $\alpha\delta$-open sets U and V such that $x \in U$ and $f^{-1}(F) \subset V$. That is, $y = f(x) \in f(U)$ and $F \subset f(V)$. Since f is strongly $\alpha\delta$-open (resp. $(Q, \alpha\delta)$-open) and bijective, $f(U)$ and $f(V)$ are disjoint $\alpha\delta$-open (resp. open) sets in . Therefore, Y is $\alpha\delta$-regular (resp. regular).

3.8. Theorem If $f : X \to Y$ is $\alpha\delta$-continuous, closed, injection and Y is regular, then X is $\alpha\delta$-regular.

Proof. Let F be a closed set in X and $x \notin F$. Since f is closed injection $f(F)$ is closed set in Y such that $f(x) \notin f(F)$. Now Y is regular, there exist disjoint open sets G and H such that $f(x) \in G$ and $f(F) \subset H$. This implies $x \in f^{-1}(G)$ and $F \subset f^{-1}(H)$. Since f is $\alpha\delta$-continuous, $f^{-1}(G)$ and $f^{-1}(H)$ are $\alpha\delta$-open sets in X. Further $f^{-1}(G) \cap f^{-1}(H) = \varphi$. Hence X is $\alpha\delta$-regular.

3.9. Theorem If $f : X \to Y$ is semi $\alpha\delta$-continuous, closed (resp. semi-closed), injection and Y is s-regular (resp. semi regular) then X is $\alpha\delta$-regular.

Proof. Let F be a closed set in X and $x \notin F$. Since f is closed (resp. semi-closed) injection $f(F)$ is closed (resp. semi-closed) set in Y such that $f(x) \notin f(F)$. Now Y is s-regular (resp. semi regular), there exist disjoint semi-open sets G and H such that $f(x) \in G$ and $f(F) \subset H$. This implies $x \in f^{-1}(G)$ and $F \subset f^{-1}(H)$. Since f is semi $\alpha\delta$-continuous $f^{-1}(G)$ and $f^{-1}(H)$ are $\alpha\delta$-open sets in X. Further $f^{-1}(G) \cap f^{-1}(H) = \varphi$. Hence X is $\alpha\delta$-regular.

3.10. Theorem If $f : X \to Y$ is strongly $\alpha\delta$-continuous, closed (resp. pre-closed), injection and Y is $\alpha\delta$-regular then X is s-regular (resp. semi regular).

Proof. Let F be a closed (semi-closed) set in X and $x \notin F$. Since f is closed (resp. pre-closed) injection $f(F)$ is closed set in Y such that $f(x) \notin f(F)$. Now Y is $\alpha\delta$-regular, there exist disjoint $\alpha\delta$-open sets G and H such that $f(x) \in G$ and $f(F) \subset H$. This implies $x \in f^{-1}(G)$ and $F \subset f^{-1}(H)$. Since f is strongly $\alpha\delta$-continuous $f^{-1}(G)$ and $f^{-1}(H)$ are open (hence semi-open) sets in X. Further $f^{-1}(G) \cap f^{-1}(H) = \varphi$. Hence X is regular (resp. semi regular).

3.11. Theorem If $f : X \to Y$ is $\alpha\delta$-irresolute, closed, injection and Y is $\alpha\delta$-regular then X is $\alpha\delta$- regular.

Proof. Let F be a closed set in X and $x \notin F$. Since f is closed injection $f(F)$ is closed set in Y such that $f(x) \notin f(F)$. Now Y is $\alpha\delta$-regular, there exist disjoint $\alpha\delta$-open sets G and H such that $f(x) \in G$ and $f(F) \subset H$. This implies $x \in f^{-1}(G)$ and $F \subset f^{-1}(H)$. Since f is $\alpha\delta$-irresolute $f^{-1}(G)$ and $f^{-1}(H)$ are $\alpha\delta$-open sets in X. Further $f^{-1}(G) \cap f^{-1}(H) = \varphi$. Hence X is $\alpha\delta$-regular.

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$M^{[x]}/G/1$ FEEDBACK QUEUE WITH THREE STAGE HETEROGENEOUS SERVICE, MULTIPLE ADAPTIVE VACATION AND CLOSED DOWN TIMES

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ABSTRACT

We consider a batch arrival queueing system with three stage heterogeneous service provided by a single server with different (arbitrary) service time distributions. Each customer undergoes three stages of heterogeneous service. As soon as the completion of third stage of service, if the customer is dissatisfied with his service, he can immediately join the tail of the original queue. The vacation period has two heterogeneous phases. After service completion of a customer the server may take a phase one Bernoulli vacation. Further, after completion of phase one Bernoulli vacation the server may take phase two optional vacation. The vacation times are assumed to be general. In addition we assume restricted admissibility of arriving batches in which not all batches are allowed to join the system at all times. The time dependent probability generating functions have been obtained in terms of their Laplace transforms and the corresponding steady state results have been obtained explicitly. Also the mean number of customers in the queue and the system are also derived. Some particular cases and numerical results are discussed.

Keywords: Heterogeneous Service, Multiple Adaptive Vacation, Closed Down Times.

1. INTRODUCTION

During the last three or four decades, queueing models with vacations had been the subject of interest to queueing theorists of deep study because of their applicability and theoretical structures in real life situations such as manufacturing and production systems, computer and communication systems, service and distribution systems, etc. The $M^{[X]}/G/1$ queue has been studied by numerous authors including Scholl and Kleinrock 1983, Gross and Harris, 1985, Doshi 1986, Kashyap and Chaudhry 1988, Shanthikumar 1988, Choi and Park 1990 and Madan 2000, 2005. Krishnakumar *et al.,* 2002 considered an M/G/1 retrial queue with additional phase of service. Madan and Anabosi 2003, have studied a single server queue with optional server vacations based on Bernoulli schedules and a single vacation policy. Madan and Choudhury 2005, have studied a single server queue with two phase of heterogeneous service under Bernoulli schedule and a general vacation time. Thangaraj and Vanitha 2010 have studied a single server $M^{[X]}/G/1$ feedback queue with two types of service having general distribution. Levy and Yechiali 1976, Baba 1986, Keilson and Servi 1986, C.Gross and C.M. Harris 1985, Takagi 1992, Borthakur and Chaudhury 1997, Cramer 1989, and many others have studied vacation queues with different vacation policies. In some queueing systems with batch arrival there is a restriction such that not all batches are allowed to join the system at all time. This policy is named restricted admissibility. Madan and Choudhury 2005 proposed anqueueing system with restricted admissibilty of arriving batches and Bernoulli schedule server vacation. In this paper, we consider a batch arrival queueing system with three stage heterogeneous service provided by a single server with different (arbitrary) service time distributions. Each customer undergoes three stage heterogeneous service. As soon as the completion of third stage of service, if the customer is dissatisfied with his service, he can immediately join the tail of the original queue as a feedback customer with probability p to repeat the service until it is successful or may depart the system with probability $1-p$ if service happens to be successful. The vacation period has two heterogeneous phases. Further, after service completion of a customer the server may take phase one vacation with probability r or return back to the system with probability $1-r$ for the next service. After the completion of phase one vacation the servermay take phase two optional vacation with probability θ or return back to the system with probability$1-\theta$. In addition we assume restricted admissibility of arriving batches in which not all batches are allowed to join the system at all times. This paper is organized as follows. 2. Supplementary variable technique. The mathematical description of our model is given in section 3. Definitions and Equations governing the system are given in section 4. The time dependent solution have been obtained in section 5. and corresponding steady state results have been

derived explicitly in section 6. Mean queue size and mean system size are computed in section

7. Some particular cases are given in section

8. Conclusion are given in section 9 respectively.

2. SUPPLEMENTARY VARIABLE TECHNIQUE

2.1. PGF of $M^x/G/1$ Model without Vacations or Closed-down time Jobs

2.2. Description of the Model and Basic Equations

Let

$$P_{1j}(x,t) = P\{N_s(t) = 1, N_q(t) = j, x \le S^0(t) \le x + dt, Y(t) = 0, j \ge 0\}$$

The basic steady state equations are

$$P_{00}(x - \Delta t, t + \Delta t) = P_{00}(x,t)(1 - \lambda \Delta t) + P_{10}(0,t)\Delta t \quad (1)$$

$$P_{10}(x - \Delta t, t + \Delta t) = P_{10}(x,t)(1 - \lambda \Delta t) + P_{11}(0,t)s(x)\Delta t + P_{00}(0,t)\lambda s(x)\Delta t \quad (2)$$

$$P_{1j}(x - \Delta t, t + \Delta t) = P_{1j}(x,t)(1 - \lambda \Delta t) + P_{1j+1}(0,t)s(x)\Delta t + \sum_{k=1}^{j} P_{1j-k}(x,t)\lambda g_k \Delta t \quad (3)$$

2.3. Queue Size Distribution

Move the first coefficients $P_{ij}(x,t)$of (1) – (3) to the left side and take the limit as $\Delta t \to 0$, we get

$$P_{00}'(x) = -\lambda P_{00}(x) + P_{10}(0) \quad (4)$$

$$P_{10}'(x) = -\lambda P_{10}(x) + P_{11}(0)s(x) + P_{00}(0)\lambda s(x) \quad (5)$$

$$P_{1j}'(x) = -\lambda P_{1j}(x) + P_{1j+1}(0)s(x) + \sum_{k=1}^{j} P_{1j-k}(x)\lambda g_k \quad (6)$$

The Laplace Stieltje's Transform of $P_{ij}(x)$ is defined as

$$\tilde{P}_{ij}(\theta) = \int_0^m e^{-\theta x} P_{ij}(x)dx$$

Therefore the Laplace Stieltje's Transform of $P_{ij}'(x)$ is given by

$$\int_0^m e^{-\theta x} P_{ij}'(x)dx = e^{-\theta x} P_{ij}(x) - \int_0^m (-\theta)e^{-\theta x} P_{ij}(x)dx = \theta \tilde{P}_{ij}(\theta) - P_{ij}(0)$$

So if $\tilde{S}(\theta)$ is the LST of the service time, the steady state queue size equations are given by

$$\theta \tilde{P}_{10}(\theta) - P_{10}(0) = \lambda \tilde{P}_{10}(\theta) - P_{11}(0)\tilde{S}(\theta) - P_{00}(0)\lambda \tilde{S}(\theta) \quad (7)$$

$$\theta \tilde{P}_{1j}(\theta) - P_{1j}(0) = \lambda \tilde{P}_{10}(\theta) - P_{1j+1}(0)\tilde{S}(\theta) - P_{00}(0)\lambda \tilde{S}(\theta) + \sum_{k=1}^{j} \tilde{P}_{1,j-k}(x)\lambda g_k \quad (8)$$

to apply the technique of Lee, H. S. [6], we define the following PGFs

$$\tilde{P}_i(z,\theta) = \sum_{j=0}^{m} \tilde{P}_{ij}(\theta)z^j, P_i(z,0) = \sum_{j=0}^{m} P_{1j}(0)z^j \quad (9)$$

Multiply Eq. (7) by z^0 and Eq. (8) by z^j $(j \ge 1)$ and take the summation from $j = 0$ tom, we have

$$\theta \sum_{j=0}^{m} \tilde{P}_{1j}(\theta)z^j - \sum_{j=0}^{m} P_{1j}(0)z^j = \lambda \sum_{j=0}^{m} \tilde{P}_{1j}(\theta)z^j - z^{-1} \sum_{j=0}^{m} \tilde{P}_{1j+1}(0)z^{j+1}\tilde{S}(\theta) - \sum_{j=1}^{m}\sum_{k=1}^{j} \tilde{P}_{1j+1}(\theta)z^{j-k}z^k\lambda g_k - \lambda \tilde{S}(\theta)P_{00}(0)z^0.$$

Using the LST in (9), we have

$$\theta \tilde{P}_1(z,\theta) - P_1(z,0) = \lambda \tilde{P}_1(z,\theta) - z^{-1}[P_1(z,0) - P_{10}(0)]\tilde{S}(\theta) - \lambda \sum_{k=1}^{m} z^k g_k \tilde{P}_1(z,\theta) - \lambda \tilde{S}(\theta)P_{00}(0) \quad (10)$$

where$P_{10} = \lambda P_{00}$ since $P_{00}'(x) = 0$ in (4)

and

$$\sum_{k=1}^{m} g_k z^k = \sum_{k=1}^{m} P(X = k)z^k = G(z).$$

Hence

$$[\theta - \lambda + \lambda G(z)]\tilde{P}_1(z,\theta) = \left(1 - z^{-1}\tilde{S}(\theta)\right)P_1(z,0) + \lambda \tilde{S}(\theta)(z^{-1} - 1)P_{00}(0) \quad (11)$$

Put $\theta = \lambda - \lambda G(z)$ in (11), we have

$$\left(\frac{z - \tilde{S}(\lambda - \lambda G(z))}{z}\right)P_1(z,0) = \lambda \tilde{S}\big(-\lambda G(z)\big)\left(\frac{z-1}{z}\right)P_{00}(0)$$

$$P_1(z,0) = \frac{\lambda \tilde{S}\big(\lambda - \lambda G(z)\big)(z-1)P_{00}(0)}{z - \tilde{S}\big(\lambda - \lambda G(z)\big)} \quad (12)$$

By substituting (12) in (11), we obtain

$$\left(\theta-\lambda+\lambda G(z)\right)\tilde{P}_1(z,\theta)$$

$$=\lambda\left(\frac{1-z}{z}\right)\left(\begin{array}{c}\left(\tilde{S}(\theta)-z\right)\\ \dfrac{\tilde{S}\left(\lambda-\lambda G(z)\right)}{z-\tilde{S}\left(\lambda-\lambda G(z)\right)}+\tilde{S}(\theta)\end{array}\right)P_{00}(0)$$

$$=\lambda\left(\frac{1-z}{z}\right)\left(\frac{z\left(\tilde{S}(\theta)-\tilde{S}\left(\lambda-\lambda G(z)\right)\right)}{z-\tilde{S}\left(\lambda-\lambda G(z)\right)}\right)P_{00}(0)$$

$$\tilde{P}_1(z,\theta)=\frac{\lambda(1-z)\left(\tilde{S}(\theta)-\tilde{S}\left(\lambda-\lambda G(z)\right)\right)}{\left(\theta-\lambda+\lambda G(z)\right)\left(z-\tilde{S}\left(\lambda-\lambda G(z)\right)\right)}$$

$$P_{00}(0) \qquad (13)$$

Let $P(z)$ be the PGF of the queue size at an arbitrary time epoch. Then $P(z)$ is the sum of the PGFs of queue size at server completion epoch and idle time epoch. So

$$P(z)=\tilde{P}_1(z,0)+P_{00}(0) \qquad (14)$$

By substituting $\theta=0$ in the equation (13), the equation (14) becomes

$$P(z)=\left(\begin{array}{c}\lambda(1-z)\\ \dfrac{\left(1-\tilde{S}\left(\lambda-\lambda G(z)\right)\right)}{\left(-\lambda+\lambda G(z)\right)}+1\\ \left(z-\tilde{S}\left(\lambda-\lambda G(z)\right)\right)\end{array}\right)P_{00}(0)$$

$$=\frac{(1-z)\left(1-\tilde{S}\left(\lambda-\lambda G(z)\right)\right)-\left(1-G(z)\right)}{(G(z)-1)\left(z-\tilde{S}\left(\lambda-\lambda G(z)\right)\right)}P_{00}(0)$$

$$P(z)=\frac{1-z\left((1-Z)-\tilde{S}\left(\lambda-\lambda G(z)\right)\right)-\left(1-G(z)\right)}{(G(z)-1)\left(z-\tilde{S}\left(\lambda-\lambda G(z)\right)\right)}$$

which represents the PGF of number of customers in queue in an arbitrary time epoch.

3. MATHEMATICAL DESCRIPTION OF THE MODEL

We assume the following to describe the equueing model of our study.

a) Customers arrive at the system in batches of variable size in a compound Poisson process and they are provided one by one service on a first come - first served basis.

Let $\lambda C_i dt(i\geq 1)$ be the first order probability that a batch of i customers arrives at the system during a short interval of time (t,t + dt], where $0\leq C_i\leq 1$ and $\sum_{i=0}^{m} C_i=1$ and $\lambda > 0$ is the arrival rate of batches.

b) (b)A single server provides three stages of service for each customer, with the service times having general distribution. Let Bi(v) and bi(v) (I =1, 2, 3) be the distribution and the density function of i stage service respectively.

c) The service time follows a general (arbitrary) distribution with distribution function $B_i(s)$ and density function $b_i(s)$. Let $\mu_i(x)dx$ be the conditional probability density of service completion during the interval $(x,x+dx]$, given that the elapsed time is x, so that

$$\mu_i(x)=\frac{b_i(x)}{1-B_i(x)}\ , \qquad i=1,2,3,\ldots$$

and therefore,

$$b_i(s)=\mu_i(s)\mathrm{e}^{-\int_0^{\mathrm{m}}\mu_i(x)dx}\ , \qquad i=1,2,3,\ldots$$

d) Moreover, after the completion of third stage of service, if the customer is dissatisfied with his service, he can immediately join the tail of the original queue as a feedback customer for receiving another service with probability . Otherwise the customer may depart forever from the system with probability $(1-p)$. Further, we do not distinguish the new arrival with feedback.

e) As soon as the third stage of service is completed, the server may take phase one Bernoulli vacation with probability r or may continue staying in the system with probability $1-r$. After completion of phase one vacation the server may take phase two optional vacation with probability θ or return back to the system with probability $1-\theta$ On returning from vacation the server starts instantly serving the customer at the head of the queue, if any.

f) The server's vacation time follows a general (arbitrary) distribution with distribution function $c_i(t)$ and density function $c_i(t)$. Let $\gamma_i(x)dx$ be the conditional probability of a completion of a vacation during the interval $(x,x+dx]$ given that the elapsed vacation time is x, so that

$\gamma_i(x)=\frac{c_i(x)}{1-c_i(x)}$, $i=1,2,\ldots$ and therefore

$$v_i(t)=\gamma_i(t)\mathrm{e}^{-\int_0^{\mathrm{t}}\gamma_i(x)dx}, i=1,2,\ldots.$$

g) The restricted admissibility of batches in which not all batches are allowed to join the

(15)

system at all times. Let $\alpha(0 \le \alpha \le 1)$ and $\beta(0 \le \beta \le 1)$ be the probability that an arriving batch will be allowed to join the system during the period of server's non-vacation period and vacation period respectively.

h) Various stochastic processes involved in the system are assumed to be independent of each other.

4. DEFINITIONS AND EQUATIONS GOVERNING THE SYSTEM

We define $P_n^{(1)}(x,t)$ = Probability that at time *t,* the server is active providing first stage of service and there are n ($n \ge 0$) customers in the queue excluding the one being served and the elapsed service time for this customer is x. Consequently $P_n^{(1)}(t) = \int_0^m P_n^{(1)}(x,t)\,dx$ denotes the probability that at time t there are n customers in the queue excluding one customer in the first stage of service irrespective of the value of x.

$P_n^{(2)}(x,t)$= Probability that at time *t,* the server is active providing second stage of service and there are n ($n \ge 0$) customers in the queue excluding the one being served and the elapsed service time for this customer is x.

Consequently $P_n^{(2)}(t) = \int_0^m P_n^{(2)}(x,t)\,dx$ denotes the probability that at time t there are n customers in the queue excluding one customer in the second stage of service irrespective of the value of$x$.

$P_n^{(3)}(x,t)$= Probability that at time *t,* the server is active providing third stage of service and there are n ($n \ge 0$)customers in the queue excluding the one being served and the elapsed service time for this customer is$x$.

Consequently $P_n^{(3)}(t) = \int_0^m P_n^{(3)}(x,t)\,dx$ denotes the probability that at time t there are n customers in the queue excluding one customer in the third stage of service irrespective of the value of$x$.

$c_n^{(1)}(x,t)$ = Probability that at time *t,* the server is under phase one vacation with elapsed vacation time x and there are $n(n \ge 0)$ customers in the queue. Consequently $c_n^{(1)}(t) = \int_0^m c_n^{(1)}(x,t)\,dx$ denotes the probability that at time t there are n customers in the queue and the server is under phase one vacation irrespective of the value of x.

$c_n^{(2)}(x,t)$ = Probability that at time *t,* the server is under phase two vacation with elapsed vacation time x and there are $n(n \ge 0)$ customers in the queue. Consequently $c_n^{(2)}(t) = \int_0^m c_n^{(2)}(x,t)\,dx$ denotes the probability that at time t there are n customers in the queue and the server is under phase two vacation irrespective of the value of x.

Q(t) = Probability that at time *t,* there are no customers in the queue and the server is idle but available in the system. The model is then, governed by the following set of

differential-difference equations:

$$\frac{\partial}{\partial x}P_0^{(1)}(x,t) + \frac{\partial}{\partial t}P_0^{(1)}(x,t) + [\lambda + \mu_1(x)]P_0^{(1)}(x,t) = \lambda(1-\alpha)P_0^{(1)}(x,t) \quad (16)$$

$$\frac{\partial}{\partial x}P_n^{(1)}(x,t) + \frac{\partial}{\partial t}P_n^{(1)}(x,t) + [\lambda + \mu_1(x)]P_n^{(1)}(x,t) = \lambda(1-\alpha)P_n^{(1)}(x,t) + \lambda\alpha\sum_{k=1}^{n} c_k\,P_{n-k}^{(1)}(x,t),$$

$$n \ge 1 \quad (17)$$

$$\frac{\partial}{\partial x}P_0^{(2)}(x,t) + \frac{\partial}{\partial t}P_0^{(2)}(x,t) + [\lambda + \mu_2(x)]P_0^{(2)}(x,t) = \lambda(1-\alpha)P_0^{(2)}(x,t) \quad (18)$$

$$\frac{\partial}{\partial x}P_n^{(2)}(x,t) + \frac{\partial}{\partial t}P_n^{(2)}(x,t) + [\lambda + \mu_2(x)]P_n^{(2)}(x,t) = \lambda(1-\alpha)P_n^{(2)}(x,t) + \lambda\alpha\sum_{k=1}^{n} c_k\,P_{n-k}^{(2)}(x,t),$$

$$n \ge 1 \quad (19)$$

$$\frac{\partial}{\partial x}P_0^{(3)}(x,t) + \frac{\partial}{\partial t}P_0^{(3)}(x,t) + [\lambda + \mu_3(x)]P_0^{(3)}(x,t) = \lambda(1-\alpha)P_0^{(3)}(x,t) \quad (20)$$

$$\frac{\partial}{\partial x}P_n^{(3)}(x,t) + \frac{\partial}{\partial t}P_n^{(3)}(x,t) + [\lambda + \mu_3(x)]P_n^{(3)}(x,t) = \lambda(1-\alpha)P_n^{(3)}(x,t) + \lambda\alpha\sum_{k=1}^{n} c_k\,P_{n-k}^{(3)}(x,t),$$

$$n \ge 1 \quad (21)$$

$$\frac{\partial}{\partial x}c_0^{(1)}(x,t) + \frac{\partial}{\partial t}c_0^{(1)}(x,t) + [\lambda + \gamma_1(x)]c_0^{(1)}(x,t) = \lambda(1-\beta)c_0^{(1)}(x,t)$$

$$\frac{\partial}{\partial x}c_n^{(1)}(x,t) + \frac{\partial}{\partial t}c_n^{(1)}(x,t) + [\lambda + \gamma_1(x)]c_n^{(1)}(x,t) = \lambda(1-\beta)c_n^{(1)}(x,t) \quad (22)$$

$$\frac{\partial}{\partial x}c_n^{(1)}(x,t) + \frac{\partial}{\partial t}c_n^{(1)}(x,t) + [\lambda + \gamma_1(x)]c_n^{(1)}(x,t) = \lambda(1-\beta)c_n^{(1)}(x,t) + \lambda\beta\sum_{k=1}^{n} c_k\,C_{n-k}^{(1)}(x,t)$$

$$, n \geq 1 \qquad (23)$$

$$\frac{\partial}{\partial x} c_0^{(2)}(x,t) + \frac{\partial}{\partial t} c_0^{(2)}(x,t)\,[\lambda + \gamma_2(x)] c_0^{(2)}(x,t) = \lambda(1-\beta) c_0^{(2)}(x,t) \qquad (24)$$

$$\frac{\partial}{\partial x} c_n^{(2)}(x,t) + \frac{\partial}{\partial t} c_n^{(2)}(x,t) + [\lambda + \gamma_2(x)] c_n^{(2)}(x,t) = \lambda(1-\beta) c_n^{(2)}(x,t) + \lambda\beta \sum_{k=1}^{n} c_k\, c_{n-k}^{(2)}(x,t),$$

$$n \geq 1 \qquad (25)$$

$$\frac{d}{dt} Q(t) + \lambda Q(t) = (1-\alpha)\lambda Q(t) + (1-\theta) \int_0^m \gamma_1(x) C_0^{(1)}(x,t)dx + \int_0^m \gamma_2(x)\, C_0^{(2)}(x,t)dx + (1-p)(1-r) \int_0^m \mu_3(x)\, P_0^{(3)}(x,t)dx \qquad (26)$$

The above equations are to be solved subject to the following boundary conditions:

$$P_n^{(1)}(0,t) = \alpha\lambda C_{n+1} Q(t) + (1-\theta) \int_0^m \gamma_1 C_{n+1}^{(1)}(x,t)dx + \int_0^m \gamma_2(x)\, C_{n+1}^{(2)}(x,t)dx + p(1-r)m + (1-p)(1-r)m, n \geq 0 \qquad (27)$$

$$P_n^{(2)}(0,t) = \int_0^m \mu_1(x) P_n^{(1)}(x,t)dx, n \geq 0 \qquad (28)$$

$$P_n^{(3)}(0,t) = \int_0^m \mu_2(x) P_n^{(2)}(x,t)dx, n \geq 0 \qquad (29)$$

$$C_n^{(1)}(0,t) = r(1-p) \int_0^m \mu_3(x) P_n^{(3)}(x,t)dx + rp, \int_0^m \mu_3(x) P_{n-1}^{(3)}(x,t)dx,$$

$$n \geq 0 \qquad (30)$$

$$C_n^{(2)}(0,t) = \theta \int_0^m \gamma_1(x)\, C_n^{(1)}(x,t)dx, n \geq 0 \qquad (31)$$

We assume that initially there are no customers in the system and the server is idle. So the initial conditions are

$$C_0^j(0) = C_0^j(0) = 0, j = 1,2, \ldots \text{ and } Q(0) = 1 \text{ and } P_n^i(0) = 0, \text{for } n = 0,1,2, \ldots., \quad i = 1,2,3 \ldots. \qquad (32)$$

5. GENERATING FUNCTIONS OF THE QUEUE LENGTH: THE TIME-DEPENDENT SOLUTION

In this section we obtain the transient solution for the above set of dfferential- difference equations.

5.1. Theorem

The system of differential difference equations to describean $M^{[X]}/G/1$ queue with three stages of heterogeneous service, feedback and Bernoulli vacation and optional server vacation with restricted admissibility are given by equations (16) to (31) with initial condition (32) and the generating functions of transient solution are given by equations (90) to (94).

Proof: We define the probability generating functions,

$$P^{(i)}(x,z,t) = \sum_{n=0}^{m} z^n\, P_n^{(i)}(x,t);\; P^{(i)}(z,t) = \sum_{n=0}^{m} z^n\, P_n^{(i)}(t), \text{for } i = 1,2,3\ldots \qquad (33)$$

$$C^{(i)}(x,z,t) = \sum_{n=0}^{m} z^n\, C_n^{(j)}(x,t);$$

$$C^{(i)}(z,t) = \sum_{n=0}^{m} z^n\, C_n^{(i)}(t), C(z) = \sum_{n=0}^{m} c_n z^n \quad \text{for } j = 1,2 \ldots \qquad (34)$$

which are convergent inside the circle given by $z \leq 1$ and define the Laplace transform of a function $f(t)$ as

$$\overline{f}(s) = \int_0^m e^{-st} f(t)dt, \;\; \mathrm{R}(s) > 0. \qquad (35)$$

Taking the Laplace transform of equations (16) to (31) and using (32), we obtain

$$\frac{\partial}{\partial_x} \overline{P}_0^{(1)}(x,s) + \left(s + \lambda\alpha + \mu_1(x)\right)$$

$$\overline{P}_0^{(1)}(x,s) = 0 \qquad (36)$$

$$\frac{\partial}{\partial_x}\overline{P}_n^{(1)}(x,s)+\big(s+\lambda\alpha+\mu_1(x)\big)\overline{P}_n^{(1)}(x,s)=\lambda\alpha\sum_{k=1}^{n}C_K\overline{P}_{n-k}^{(1)}(x,s),\quad n\geq 1 \tag{37}$$

$$\frac{\partial}{\partial_x}\overline{P}_0^{(2)}(x,s)+\big(s+\lambda\alpha+\mu_2(x)\big)\overline{P}_0^{(2)}(x,s),n=0 \tag{38}$$

$$\frac{\partial}{\partial_x}\overline{P}_n^{(2)}(x,s)+(s+\lambda\alpha+\mu_2(x))\overline{P}_n^{(2)}(x,s)=\lambda\alpha\sum_{k=1}^{n}c_k\overline{P}_{n-k}^{(2)}(x,s),n\geq 1 \tag{39}$$

$$\frac{\partial}{\partial_x}\overline{P}_0^{(3)}(x,s)+(s+\lambda\alpha+\mu_3(x))\overline{P}_0^{(3)}(x,s)=0 \tag{40}$$

$$\frac{\partial}{\partial_x}\overline{P}_n^{(3)}(x,s)+\big(s+\lambda\alpha+\mu_3(x)\big)\overline{P}_n^{(3)}(x,s)=\lambda\alpha\sum_{k=1}^{n}c_k\overline{P}_{n-k}^{(2)}(x,s),n\geq 1 \tag{41}$$

$$\frac{\partial}{\partial_x}\overline{C}_0^{(1)}(x,s)+\big(s+\lambda\beta+\gamma_1(x)\big)\overline{C}_0^{(1)}(x,s)=0 \tag{42}$$

$$\frac{\partial}{\partial_x}\overline{C}_n^{(1)}(x,s)+(s+\lambda\beta+\gamma_1(x))\overline{C}_n^{(1)}(x,s)=\lambda\beta\sum_{k=1}^{n}c_k\overline{C}_{n-k}^{(1)}(x,s),n\geq 1 \tag{43}$$

$$\frac{\partial}{\partial_x}\overline{C}_0^{(2)}(x,s)+\big(s+\lambda\beta+\gamma_2(x)\big)\overline{C}_0^{(2)}(x,s)=0 \tag{44}$$

$$\frac{\partial}{\partial_x}\overline{C}_n^{(2)}(x,s)+\big(s+\lambda\beta+\gamma_2(x)\big)\overline{C}_n^{(2)}(x,s)=\lambda\beta\sum_{k=1}^{n}c_k\overline{C}_{n-k}^{(2)}(x,s),\quad n\geq 1 \tag{45}$$

$$[s+\lambda\alpha]\overline{Q}(s)=+(1-\theta)\int_0^m\gamma_1(x)\overline{C}_0^{(1)}(x,s)dx+\int_0^m(1-p)(-r)\int_0^m\mu_3(x)\overline{P}_0^{(3)}(x,s)dx \tag{46}$$

$$\overline{P}_0^{(1)}(0,s)=\alpha\lambda c_n+1\overline{Q}(s)(1-\theta)\int_0^m\gamma_1(x)\overline{C}_{n+1}^{(1)}(x,s)dx+\int_0^m\gamma_2(x)\overline{C}_{n+1}^{(2)}(x,s)dx+p(1)-r)\int_0^m\mu_3(x)\overline{P}_n^{(3)}(x,s)dx+(1-p)(1-r)\int_0^m\overline{P}_n^{(3)}(x,s)\mu_3(x)dx,n\geq 0 \tag{47}$$

$$\overline{P}_n^{(2)}(0,s)=\int_0^m\mu_1(x)\overline{P}_n^{(1)}(x,s)dx,\quad n\geq 0 \tag{48}$$

$$\overline{P}_n^{(3)}(0,s)=\int_0^m\mu_2(x)\overline{P}_n^{(2)}(x,s)dx,n\geq 0 \tag{49}$$

$$\overline{C}_n^{(1)}(0,s)=r(1-p)\int_0^m\mu_3(x)\overline{P}_n^{(3)}(x,s)(x)dx+rp\int_0^m\mu_3(x)\overline{P}_{n-1}^{(3)}(x,s)(x)dx,\quad n\geq 0 \tag{50}$$

$$\overline{C}_n^{(2)}(0,s)=\theta\int_0^m\gamma_1(x)\overline{C}_n^{(1)}(x,s)dx,\quad n\geq 0 \tag{51}$$

Now multiplying equations (37), (39), (41), (43) and (45) by z^n and summing over n from 1 to$m$, adding to equations (36), (38), (40), (42), (44) and using the generating functions defined in (33) and (34) we get

$$\frac{\partial}{\partial_x}\overline{P}_0^{(1)}(x,z,s)+\big[s+\lambda\alpha\big(1-C(z)\big)+\mu_1(x)\big]\overline{P}^{(1)}(x,z,s)=0 \tag{52}$$

$$\frac{\partial}{\partial_x}\overline{P}_0^{(2)}(x,z,s)+[s+\lambda\alpha(1-C(z))+\mu_2(x)]\overline{P}^{(2)}(x,z,s)=0 \tag{53}$$

$$\frac{\partial}{\partial_x}\overline{P}_0^{(3)}(x,z,s)+\big[s+\lambda\alpha\big(1-C(z)\big)+\mu_3(x)\big]\overline{P}^{(3)}(x,z,s)=0 \tag{54}$$

$$\frac{\partial}{\partial_x}\overline{C}_0^{(1)}(x,z,s)+[s+\lambda\beta(1-C(z))+\gamma_1(x)]\overline{C}^{(1)}(x,z,s)=0 \tag{55}$$

$$\frac{\partial}{\partial_x}\overline{C}_0^{(2)}(x,z,s) + \left[s+\lambda\beta\left(1-C(z)\right)+\gamma_2(x)\right]\overline{C}^{(2)}(x,z,s) = 0 \quad (56)$$

For the boundary conditions, we multiply both sides of equation (47) by z^n sum over n from 0 to m, and use the equation (33) and (34) to get

$$z\,\overline{P}^{(1)}(0,z,s) = \alpha\lambda c(z)\overline{Q}(s) + (1-\theta)\int_0^m \gamma_1(x)\overline{C}^{(1)}(x,z,s)dx + \int_0^m \gamma_2(x)\overline{C}^{(2)}(x,z,s)dx + pz(1-r)\int_0^m \mu_3(x)\overline{P}^{(3)}(x,z,s)dx + (1-p)(1-r)\int_0^m \mu_3(x)\overline{P}^{(3)}(x,z,s)dx - (1-\theta)\int_0^m \gamma_1(x)\overline{C}_0^{(1)}(x,s)dx - \int_0^m \gamma_2(x)\overline{C}_0^{(2)}(x,s)dx - (1-p)(1-r)\int_0^m \mu_3(x)\overline{P}_0^{(3)}(x,s)dx \quad (57)$$

Using equation (31), equation (40) becomes

$$z\,\overline{P}^{(1)}(0,z,s)1 + [\lambda\alpha(C(z)-1)s]\mathrm{Q(s)} + (1-\theta)\int_0^m \gamma_1(x)\overline{C}^{(1)}(x,z,s)dx + \int_0^m \gamma_2(x)\overline{C}^{(2)}(x,z,s)dx + (pz+1-p)(1-r)\int_0^m \mu_3(x)\overline{P}^{(3)}(x,z,s)dx. \quad (58)$$

Performing similar operation on equations (48), (49), (50) and (51) we get,

$$\overline{P}^{(2)}(0,z,s) = \int_0^m \mu_1(x)\overline{P}^{(1)}(x,z,s)dx \quad (59)$$

$$\overline{P}^{(3)}(0,z,s) = \int_0^m \mu_2(x)\overline{P}^{(2)}(x,z,s)dx \quad (60)$$

$$\overline{C}^{(1)}(0,z,s) = r(1-p+pz)\int_0^m \mu_3(x)\overline{P}^{(3)}(x,z,s)dx \quad (61)$$

$$\overline{C}^{(2)}(0,z,s) = \theta\int_0^m \gamma_1(x)\overline{C}^{(1)}(x,z,s)dx \quad (62)$$

Integrating equation (52) between 0 to x, we get

$$\overline{P}^{(1)}(0,z,s) = \overline{P}^{(1)}(x,z,s)\, e^{-\left[s+\lambda\alpha\left(1-C(z)\right)\right]x-\int_0^x \mu_1(t)dt} \quad (63)$$

Where $P^{(1)}(0,z,s)$ is given by equation (58).

Again integrating equation (63) by parts with respect to x yields,

$$\overline{P}^{(1)}(z,s) = \overline{P}^{(1)}(0,z,s)\begin{bmatrix}(1-\overline{B}_1)\\ \dfrac{\left(s+\lambda\alpha\left(1-C(z)\right)\right)}{s+\lambda\alpha\left(1-C(z)\right)}\end{bmatrix} \quad (64)$$

Where

$$\overline{B}_1\left(s+\lambda\alpha\left(1-C(z)\right)\right) = \int_0^m e^{-\left[s+\lambda\alpha\left(1-C(z)\right)\right]x}dB_1(x) \quad (65)$$

is the Laplace-Stieltjes transform of the first stage service time $B_1(x)$ Now multiplying both sides of equation (63) by $\mu_1(x)$

and integrating over x we obtain

$$\int_0^m \overline{P}^{(1)}(0,z,s)dx = \overline{P}^{(1)}(0,z,s)\overline{B}_1 [s+\lambda\alpha\left(1-C(z)\right)] \quad (66)$$

Similarly, on integrating equations (53) to (56) from 0 tox, we get

$$\overline{P}^{(2)}(x,z,s) = \overline{P}^{(2)}(0,z,s)\, e^{-\left[s+\lambda\alpha\left(1-C(z)\right)\right]x-\int_0^x \mu_2(t)dt} \quad (67)$$

$$\overline{P}^{(3)}(x,z,s)\ \overline{=P}^{(3)}(0,z,s)e^{-\left[s+\lambda\alpha\left(1-C(z)\right)\right]x-\int_0^x \mu_3(t)dt} \quad (68)$$

$$\overline{C}^{(1)}(x,z,s) = \overline{C}^{(1)}(0,z,s)e^{-\left[s+\lambda\beta\left(1-C(z)\right)\right]x-\int_0^x \gamma_1(t)dt} \quad (69)$$

$$\overline{C}^{(2)}(x,z,s) = \overline{C}^{(2)}(0,z,s)e^{-\left[s+\lambda\beta\left(1-C(z)\right)\right]x-\int_0^x \gamma_2(t)dt} \quad (70)$$

Where $\overline{P}^{(2)}(x,z,s)$,$\overline{P}^{(3)}(x,z,s)$,$\overline{C}^{(1)}(x,z,s)$, and $\overline{C}^{(2)}(x,z,s)$ are given by equations (59) to (62). Again integrating equations

(67) to (70) by parts with respect to x yields,

$$\overline{P}^{(2)}(z,s) = \overline{P}^{(2)}(0,z,s)\left[\frac{(1-\overline{B}_2)}{\left(s+\lambda\alpha\left(1-C(z)\right)\right)}{s+\lambda\alpha\left(1-C(z)\right)}\right] \quad (71)$$

$$\overline{P}^{(3)}(z,s) = \overline{P}^{(3)}(0,z,s)\left[\frac{(1-\overline{B}_3)}{\left(s+\lambda\alpha\left(1-C(z)\right)\right)}{s+\lambda\alpha\left(1-C(z)\right)}\right] \quad (72)$$

$$\overline{C}^{(1)}(z,s) = \overline{C}^{(1)}(0,z,s)\left[\frac{(1-\overline{C}_1)}{\left(s+\lambda\beta\left(1-C(z)\right)\right)}{s+\lambda\beta\left(1-C(z)\right)}\right] \quad (73)$$

$$\overline{C}^{(2)}(z,s) = \overline{C}^{(2)}(0,z,s)\left[\frac{(1-\overline{C}_2)}{\left(s+\lambda\beta\left(1-C(z)\right)\right)}{s+\lambda\beta\left(1-C(z)\right)}\right] \quad (74)$$

Where

$$\overline{B}_2\left(s+\lambda\alpha\left(1-C(z)\right)\right) = \int_0^m e^{-[s+\lambda\alpha(1-C(z))]x}dB_2(x) \quad (75)$$

$$\overline{B}_3\left(s+\lambda\alpha\left(1-C(z)\right)\right) = \int_0^m e^{-[s+\lambda\alpha(1-C(z))]x}dB_3(x) \quad (76)$$

is the Laplace-Stieltjes transform of the second and third stage service time $B_2(x)$ and $B_3(x)$ respectively. Now multiplying both sides of equation (68) by $\mu_2(x)$ and (69) by $\mu_3(x)$ and integrating over x we obtain

$$\int_0^m \overline{P}^{(2)}(x,z,s)\mu_2(x) = \overline{P}^{(2)}(0,z,s)\overline{B}_2\left[s+\lambda\alpha\left(1-C(z)\right)\right] \quad (77)$$

$$\int_0^m \overline{P}^{(3)}(x,z,s)\mu_3(x) = \overline{P}^{(3)}(0,z,s)\overline{B}_3\left[s+\lambda\alpha\left(1-C(z)\right)\right] \quad (78)$$

and

$$\overline{C}_1(s+\lambda\beta\left(1-C(z)\right) = \int_0^m e^{-[s+\lambda\beta(1-C(z))]x}dC_1(x) \quad (79)$$

$$\overline{C}_2(s+\lambda\beta\left(1-C(z)\right) = \int_0^m e^{-[s+\lambda\beta(1-C(z))]x}dC_2(x) \quad (80)$$

is the Laplace-Stieltjes transform of the vacation time $\overline{C}_1(x)$ and $\overline{C}_2(x)$) Now multiplying both sides of equation (70) by $\gamma_1(x)$

and (71) by $\gamma_2(x)$ and integrating over x we obtain

$$\int_0^m \overline{C}^{(1)}(x,z,s)\gamma_1(x) = \overline{C}^{(1)}(0,z,s)\overline{C}_1\left[s+\lambda\beta\left(1-C(z)\right)\right] \quad (81)$$

$$\int_0^m \overline{C}^{(2)}(x,z,s)\gamma_2(x) = \overline{C}^{(2)}(0,z,s)\overline{C}_2\left[s+\lambda\beta\left(1-C(z)\right)\right] \quad (82)$$

Using equation (66), equation (59) reduces to

$$\overline{P}^{(2)}(0,z,s) = \overline{P}^{(1)}(0,z,s)\overline{B}_1(R) \quad (83)$$

Now using equations (77) and (68) in (60), we get

$$\overline{P}^{(3)}(0,z,s) = \overline{P}^{(1)}(0,z,s)\overline{B}_1(R)\overline{B}_2(R) \quad (84)$$

By using equations (78) and (83) in (61), we get

$$\overline{C}^{(1)}(0,z,s) = r(+pz)\overline{B}_1(R)\overline{B}_2(R)\overline{B}_3(R)\overline{P}^{(1)}(0,z,s) \quad (85)$$

Using equations (81) and (85), we can write equation (62) as

$$\overline{C}^{(2)}(0,z,s) = \theta r(1-p - pz)\overline{B}_1(R)\overline{B}_2(R)\overline{B}_3(R)\overline{C}_1(T)\overline{P}^{(1)}(0,z,s) \quad (86)$$

Now using equations (78), (79) and (82), equation (58) becomes

$$z\overline{P}^{(1)}(0,z,s) = 1 + [\lambda\alpha(C(z)-1) - S]\overline{Q}(s) + 1 - \theta)\overline{C}_1(T)\overline{C}^{(1)}(0,z,s) + \overline{C}_2(T)\overline{C}^{(2)}(0,z,s) + (pz+1-p)(1 - r)\overline{B}_3(R)\overline{P}^{(3)}(0,z,s) \quad (87)$$

Similarly using equations (84), (85) and (86), equation (87) reduces to

$$\overline{P}^{(1)}(0,z,s) = \frac{1+[\lambda\alpha(C(z)-1)-s]\overline{Q}(s)}{DR} \quad (88)$$

Where

$$DR = z-(1-p+pz)\overline{B}_1(R)\overline{B}_2(R)\overline{B}_3(R)\left[1-r+r\overline{C}_1(T)\left(1-\theta+\theta\overline{C}_2(T)\right)\right] \quad (89)$$

$$R = s+\lambda\alpha(1-C(z)) \text{ and } T = s+\lambda\beta(1-C(z)).$$

Substituting the equations (83), (84), (85) and (88) into equations (64), (71), (72), (73) and (74) we get

$$\overline{P}^{(1)}(z,s) = \frac{\left[(1-s\overline{Q}(s)\right]+\lambda\alpha(C(z)-1)\overline{Q}(s)}{DR}\frac{[1-\overline{B}_1(R)]}{R}$$

$$\overline{P}^{(2)}(z,s) = \frac{(\overline{B}_1(R)\left[(1-s\overline{Q}(s)\right])+\lambda\alpha(C(z)-1)\overline{Q}(s)}{DR}\frac{\left[1-\overline{B}_2(R)\right]}{R} \quad (91)$$

$$\overline{P}^{(3)}(z,s) = \frac{(\overline{B}_1(R)\overline{B}_2(R)\left[(1-s\overline{Q}(s)\right])+\lambda\alpha(C(z)-1)\overline{Q}(s)}{DR}\frac{\left[1-\overline{B}_3(R)\right]}{R}$$

$$\overline{C}^{(1)}(z,s) = \frac{(r(1-p+pz))}{DR}\overline{B}_1(R)\overline{B}_2(R)\overline{B}_3(R)\left[(1-s\overline{Q}(s))+\lambda\alpha(C(z)-1)\overline{Q}(s)\right]\frac{[1-\overline{C}_1(T)]}{T} \quad (93)$$

$$\overline{C}^{(2)}(z,s) = \frac{\theta r(1-p+pz)\overline{B}_1(R)\overline{B}_2(R)\overline{B}_3(R)\overline{C}_1(T)}{DR}\left[(1-s\overline{Q}(s))+\lambda\alpha(C(z)-1)\overline{Q}(s)\right]\frac{[1-\overline{C}_1(T)]}{T} \quad (94)$$

where DR is given by equation (89). Thus $\overline{P}^{(1)}(z,s), \overline{P}^{(2)}(z,s), \overline{P}^{(3)}(z,s), \overline{C}^{(1)}(z,s)$ and $\overline{C}^{(2)}(z,s)$ are completely determined from equations (90) to (94) which completes the proof of the theorem

6. THE STEADY STATE RESULTS

In this section, we shall derive the steady state probability distribution for our queueing model. To define the steady probabilities we suppress the argument t wherever it appears in the time-dependent analysis. This can be obtained by applying the well-known Tauberian property,

$$\lim_{s\to m} s\,\overline{f}(s) = \lim_{t\to m} f(t) \quad (95)$$

In order to determine Thus $\overline{P}^{(1)}(z,s), \overline{P}^{(2)}(z,s), \overline{P}^{(3)}(z,s), \overline{C}^{(1)}(z,s)$ and $\overline{C}^{(2)}(z,s)$ completely, we have yet to determine the unknown Q which appears in the numerators of the right hand sides of equations (90) to (94). For that purpose, we shall use the normalizing condition

$$P^{(1)}(1)+P^{(2)}(1)+P^{(3)}(1)+C^{(1)}(1)+C^{(2)}(1)+Q = 1 \quad (96)$$

5.2. Theorem

The steady state probabilities for an $M^{[X]}/G/1$ feedback queue with three stage heterogeneous service, feedback, Bernoulli vacation and optional server vacation with restricted admissibility are given by

$$P^{(1)}(1) = \frac{\lambda\alpha E(I)E(B_1)Q}{dr} \quad (97)$$

$$P^{(2)}(1) = \frac{\lambda\alpha E(I)E(B_2)Q}{dr} \quad (98)$$

$$P^{(3)}(1) = \frac{\lambda\alpha E(I)E(B_3)Q}{dr} \quad (99)$$

$$C^{(1)}(1) = \frac{\lambda\alpha r E(I)E(C_1)Q}{dr} \quad (100)$$

$$C^{(2)}(1) = \frac{\lambda\alpha r\theta E(I)E(C_2)Q}{dr} \quad (101)$$

where

$$dr = 1-p-\lambda E(I)\left[\alpha\left(E(B_1)+E(B_2)E(B_3)\right)+r\beta E(C)\right], \quad (102)$$

And $E(C) = E(C_1)+\theta E(C_2)$.

$P^{(1)}(1), P^{(2)}(1), P^{(3)}(1), C^{(1)}(1) C^{(2)}(1)$ *and* Q *are the steady state probabilities that the server is providing first stage of service, second stage of service, third stage of service, server under phase one and server under phase two vacation, server under idle respectively without regard to the number of customers in the system.*

Proof: Multiplying both sides of equations (90) to (94) by s, taking limit as $s \to 0$, applying property (95) and simplifying, we obtain

$$P^{(1)}(z)\ (\lambda\alpha(C(z)-1)) = \frac{[1-\overline{B}_1(f_1(z))]Q}{f_1(z)D(z)} \quad (103)$$

$$P^{(2)}(z)\ (\lambda\alpha(C(z)-1)\overline{B}_1(f_1(z))) = \frac{[1-\overline{B}_2(f_1(z))]Q}{f_1(z)D(z)} \quad (104)$$

$$P^{(3)}(z)\ (\lambda\alpha(C(z)-1))\ \overline{B}_1(f_1(z))\overline{B}_2(f_1(z)) = \frac{[1-\overline{B}_3(f_1(z))]Q}{f_1(z)D(z)} \quad (105)$$

$$C^{(1)}(z)\ (\lambda\alpha r\binom{1-p+pz}{(C(z)-1}) = \frac{\overline{B}(z)[1-\overline{C}_1(f_3(z))]Q}{f_3(z)D(z)} \quad (106)$$

$$C^{(2)}(z)\ \begin{pmatrix}\lambda\alpha r\theta\\ \binom{1-p+pz}{(C(z)-1)}\\ \overline{B}(z)\,\overline{C}_1(f_3(z))\end{pmatrix}[1-\overline{C}_2(f_3(z))]Q = \frac{}{f_3(z)D(z)} \quad (107)$$

Where

$$D(z) = z-(1-p+pz)\overline{B}_1(f_1(z))\overline{B}_2(f_1(z))\overline{B}_3(f_1(z))\left[1-r+r\overline{C}_1(f_2(z))\left(1-\theta+\theta\overline{C}_2(f_2(z))\right)\right],$$

$$\overline{B}(z) = \overline{B}_1(f_1(z))\overline{B}_1(f_1(z))\overline{B}_3(f_1(z)), f_1(z) = \lambda\alpha(1-C(z)),$$

$$and f_2(z) = \lambda\beta(1-C(z)).$$

Let $W_q(z)$ denote the probability generating function of the queue size irrespective of the state of the system. Then adding equations (103) to (107) we obtain

$$W_q(z) = P^{(1)}(z)+P^{(2)}(z)+P^{(3)}(z)+C^{(1)}(z)+C^{(2)}(z)$$

$$W_q(z) = \frac{\lambda\alpha(C(z)-1)}{f_1(z)D(z)}\left[1-\overline{B}_1(f_1(z))\right]Q + \frac{\lambda\alpha(C(z)-1)\overline{B}_1(f_1(z))}{f_1(z)D(z)}\left[1-\overline{B}_2(f_1(z))\right]Q + \frac{\lambda\alpha(C(z)-1)\overline{B}_1(f_1(z))\overline{B}_2(f_1(z))}{f_1(z)D(z)}\left[1-\overline{B}_3(f_1(z))\right]Q + \frac{\lambda\alpha r\binom{1-p+pz}{(C(z)-1}\overline{B}(z)}{f_2(z)D(z)}\left[1-\overline{C}_1(f_2(z))\right]Q + \frac{\lambda\alpha r\theta\binom{1-p+pz}{(C(z)-1}\overline{B}(z)\overline{C}_1(f_2(z))}{f_2(z)D(z)}\left[1-\overline{C}_2(f_2(z))\right]Q \quad (108)$$

We see that for $z=1$, $W_q(1)$ is indeterminate of the form $\frac{0}{0}$. Therefore, we apply L'Hopital's rule and on simplifying we obtain the result (109), where $C(1)=1, C'(1)=E(I)$ is mean batch size of the arriving customers, $-\overline{B_i'}(0)=E(B_i), -\overline{C_j'}(0) = E(C_j), i=1,2,3,\ldots\ and\ j=1,2,\ldots$

$$W_q(1) = \frac{\lambda\alpha C'(1)\left[E(B_1)+E(B_2)E(B_3)+rE(C)\right]}{dr} \quad (109)$$

wheredr is given by equation (102). Therefore adding Q to equation (109),equating to 1 and simplifying, we get

$$Q = 1-\rho \quad (110)$$

and hence the utilization factor ρ of the system is given by

$$\rho = \frac{\alpha\lambda E(I)\left[E(B_1)+E(B_2)E(B_3)+rE(C)\right]}{d1-p-r\lambda E(I)(\beta-\alpha)E(C)]} \quad (111)$$

where$\rho<1$ is the stability condition under which the steady state exists. Equation (110) gives the probability that the server is idle. Substituting Q from (110) into (108), we have completely and explicitly determined $W_q(z)$,the probability generating function of the queue size.

7. THE MEAN QUEUE SIZE AND THE MEAN SYSTEM SIZE

Let L_q denote the mean number of customers in the queue under the steady state. Then

$$L_q = \frac{d}{dz} W_q(z) \text{at } z = 1$$

Since this formula gives $0/0$ form, then we write $W_q(z)$ given in (93) as $W_q(z) = \frac{N(z)}{D(z)}$ where $N(z)$ and $D(z)$ are numerator and denominator of the right hand side of (93) respectively. Then we use

$$L_q = \lim_{z\to 1} \frac{d}{dz} W_q(z)$$
$$= \lim_{z\to 1} \frac{1}{\beta} \begin{bmatrix} (\mathrm{D}'(1)N''(1)) \\ -N'(1)\mathrm{D}''(1) \\ 2(\mathrm{D}'(1))^2 \end{bmatrix} Q \quad (112)$$

where primes and double primes in (112) denote first and second derivative at $z = 1$, respectively. Carrying out the derivative at $z = 1$ we have

$$N'(1) = \lambda\alpha\beta E(I)[E(B_1) + E(B_2) + E(B_3) + rE(C)] \quad (113)$$

$$N''(1) = \lambda^2\beta\alpha\big(E(I)\big)^2[\alpha E(B_1^2) + E(B_2^2) + E(B_3^2) + \beta r E(C_1^2) + \theta E(C_2^2))] + \lambda\alpha\beta E\big(I(I-1)\big)$$

$$[E(B_1) + E(B_2) + E(B_3) + rE(C)] + 2\lambda^2\beta\alpha\big(E(I)\big)^2$$

$$[\alpha E(B_1)((E(B_2) + E(B_3)) + \alpha E(B_2)E(B_3) + \beta r\theta E(C_1)E(C_2)] + 2\lambda^2\beta\alpha^2 r\big(E(I)\big)^2 E(C)$$

$$\mathrm{x}[E(B_1) + E(B_2) + E(B_3)] + 2\lambda r\alpha\beta p E(I)E(C) \quad (114)$$

$$D'(1) = 1 - p - \lambda E(I)\begin{bmatrix}(\alpha E(B_1) + E(B_2)) \\ +E(B_3) + r\beta E(C)\end{bmatrix} \quad (115)$$

$$\mathrm{D}''(1) = \lambda\big[2PE(I) + E\big(I(I-1)\big)\big][\alpha(E(B_1) + \big(E(B_2) + E(B_3)\big) + r\beta E(C)\big] - 2\lambda^2\beta\alpha r\big(E(I)\big)^2 E(C)[E(B_1) + E(B_2) + E(B_3)] - \lambda^2\big(E(I)\big)^2$$

$$\mathrm{x}[\alpha^2(E(B_1^2) + E(B_2^2) + E(B_3^2) + \beta^2 rE(C_1^2) + \theta E(C_2^2)] - 2\lambda^2\big(E(I)\big)^2$$

$$\mathrm{x}\left[\alpha E(B_1)\big(\big(E(B_2) + E(B_3)\big) + \alpha E(B_2)E(B_3) + \beta^2 r\theta E(C_1)E(C_2)\right] \quad (116)$$

where $E(C^2)$, are the second moment of the vacation time, $E\big(I(I-1)\big)$ is the second factorial moment of the batch size of arriving customers. Then if we substitute the values $N'(1), N''(1), D'(1), D''(1)$from equations (113) to (101) into equations (112) we obtain Lq in the closed form.

Further, we find the mean system size L using Little's formula. Thus we have

$$L = L_q + \rho \quad (117)$$

where L_q has been found by equation (112) and ρ *is* obtained from equation (111).

8. PARTICULAR CASE

Case 1: No feedback, no optional vacation and no restricted admissibility.

Put $p = 0,\ \theta = 0, and\ \alpha = \beta = 1$in the main results, we get

$$Q = 1 - \rho \quad (118)$$

$$\rho = \lambda E(I)[E(B_1) + E(B_2) + E(B_3) + rE(C_1)] \quad (119)$$

$$N'(1) = \lambda E(I)[E(B_1) + E(B_2) + E(B_3) + rE(C_1)] \quad (120)$$

$$N''(1) = \lambda^2\big(E(I)\big)^2[E(B_1^2) + E(B_2^2) + rE(C_1^2)] + \lambda E\big(I(I-1)\big)[E(B_1) + E(B_2) + E(B_3) + rE(C_1)] + 2\lambda^2\big(E(I)\big)^2[E(B_1)(E(B_2) + E(B_3)) + E(B_2)E(B_3)] + 2\lambda^2 r\big(E(I)\big)^2 E(C_1)[E(B_1) + E(B_2) + E(B_3)] \quad (121)$$

$$D'(1) = -\lambda E(I)[E(B_1) + E(B_2) + E(B_3) + rE(C_1)] \quad (122)$$

$$D''(1) = -\lambda E\big(I(I-1)\big)[E(B_1) + E(B_2) + E(B_3) + rE(C_1)] - 2\lambda^2 r\big(E(I)\big)^2 E(C_1)[E(B_1) + E(B_2) + E(B_3)] - \lambda^2\big(E(I)\big)^2[E(B_1^2) + E(B_2^2) + E(B_3^2) + rE(C_1^2)] - 2\lambda^2\big(E(I)\big)^2\big[E(B_1)\big(E(B_2) + E(B_3)\big) + E(B_2)E(B_3)\big] \quad (123)$$

Then, if we substitute the values $N'(1),\ N''(1), D'(1), D''(1)$from equations (120) to (123) into equations (112), we obtain Lq in the closed form.

Case 2: The service and vacation times are exponential.

Put $p = 0,\ \theta = 0,\ \alpha = \beta = 1$ in the main results. The most commondistribution for the service and vacation times are the exponential distribution.

For this distribution, the exponential service rate$\mu_i > 0$ and the exponential vacation rate

$\gamma_j > 0$, for $i = 0,1,2,3,\ldots$and $j = 0,1,2,\ldots$ then we have

$$Q = 1 - \rho \tag{124}$$

$$\rho = \frac{\lambda E(I)}{\mu_1\mu_2\mu_3\gamma_1}\begin{bmatrix}(\mu_3\gamma_1(\mu_2+\mu_1))\\+\mu_1\mu_2(\gamma_1+r\mu_3)\end{bmatrix} \tag{125}$$

$$N'(1) = \lambda E(I)[\mu_3\gamma_1(\mu_2+\mu_1) + \mu_1\mu_2(\gamma_1+r\mu_3)] \tag{126}$$

$$\begin{aligned}N''(1) &= 2\lambda^2\big(E(I)\big)^2[\mu_3^2\gamma_1^2(\mu_2^2+\mu_1^2)+\mu_1^2\mu_2^2(\mu_3^2 + r\gamma_1^2)]\\ &+\lambda E\big(I(I-1)\big)\mu_1\mu_2\mu_3\gamma_1[\mu_3\gamma_1(\mu_2+\mu_1)+\mu_1\mu_2(\gamma_1 + r\mu_3)] - 2\lambda^2\big(E(I)\big)^2\mu_1\mu_2\mu_3\gamma_1^2[\mu_1+\mu_2+\mu_3]\\ &+ 2\lambda^2 r\mu_1\mu_2\mu_3\gamma_1\big(E(I)\big)^2[\mu_3(\mu_1+\mu_2) + \mu_1\mu_2]\end{aligned} \tag{127}$$

$$\begin{aligned}D'(1) &= \mu_1\mu_2\mu_3\gamma_1\\ &-\lambda E(I)\begin{bmatrix}(\mu_3\gamma_1(\mu_2+\mu_1))\\+\mu_1\mu_2(\gamma_1+r\mu_3)\end{bmatrix}\end{aligned} \tag{128}$$

$$\begin{aligned}D''(1) &= -\lambda E\big(I(I-1)\big)\gamma_1\mu_1\mu_2\mu_3[\mu_3\gamma_1(\mu_2+\mu_1) + \mu_1\mu_2(\gamma_1+r\mu_3)]\\ &-2\lambda^2 r\gamma_1\mu_1\mu_2\mu_3\big(E(I)\big)^2[\mu_2\mu_3+\mu_1\mu_{3+}\mu_1\mu_2]\\ &-2\lambda^2\big(E(I)\big)^2[\mu_3^2\gamma_1^2(\mu_2^2+\mu_1^2)+\mu_1^2\mu_2^2(\gamma_1^2+r\mu_3^2)]\\ &-2\lambda^2\big(E(I)\big)^2\gamma_1^2\mu_1\mu_2\mu_3[\mu_1+\mu_2 + \mu_3]\end{aligned} \tag{129}$$

Then, if we substitute the values $N'(1)$, $N''(1), D'(1), D''(1)$from equations (126) to (129) into equations (97), we obtain L_q in the closed form.

9. CONCLUSION

In this paper we have studied a batch arrival, three stage heterogeneous service, feedback with Bernoulli vacation and optional server vacation. This paper clearly analyzes the transient solution, steady state results. If the customer is not satisfied with the service, again he can join the tail of the queue and get the regular service.

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ON GENERALIZED GRILL CONTINUOUS FUNCTIONS

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ABSTRACT

In this paper, We introduce a new class of continuous functions namely g-G-continuousfunctions, g-G-irresolute and study some of their properties in topological spaces.

Keywords: g-G-continuous, g-G-irresolute

1. INTRODUCTION

In 1970, Levine first introduced the concept of generalized closed (briefly, g-closed) sets were defined and investigated. The idea of grill on a topological space was first introduced by Choquet in 1947. It is observed from literature that the concept of grills is a powerful supporting tool, like nets and filters, in dealing with many topological concept quite effectively. In 2007, Roy and Mukherjee defined and studied a typical topology associated rather naturally to the existing topology and a grill on a given topological space. The aim of this paper is to introduce g-G-continuous and g-G-irresolute and investigate the relations of g-G-continuous functions between such functions.

2. PRELIMINARIES

Throughout this paper, (X, τ) (or X) represent a topological space on which no separation axioms are assumed unless otherwise mentioned. For a subset A of a space X, cl(A) and int(A) denote the closure of A and the interior of A, respectively. The power set of X will be denoted by $\wp(X)$. A collection G of a nonempty subsets of a space X is called a grill (Andrijevic, 1986) on X if

(1) $A \in G$ and $A \subseteq B \Rightarrow B \in G$,

(2) $A, B \subseteq X$ and $A \cup B \in G \Rightarrow A \in G$ or $B \in G$.

For any point x of a topological space (X, τ), $\tau(x)$ denote the collection of all open neighbourhoods of x.

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

2.1 Definition (Arya and Nour, 1990). Let (X, τ) be a topological space and G be a grill on X. The mapping

$\Phi : \wp(X) \rightarrow \wp(X)$, denoted by $\Phi_G(A, \tau)$ for $A \in \wp(X)$ or simply $\Phi(A)$ called the operator associated with the grill G and the topology τ and is defined by

$\Phi_G(A) = \{x \in X \mid A \cap U \in G, \forall U \in \tau(x)\}$.

Let G be a grill on a space X. Then a map $\Psi : \wp(X) \rightarrow \wp(X)$ is defined by $\Psi(A) = A \cup \Phi(A)$, for all $A \in \wp(X)$. The map Ψ satisfies Kuratowski closure axioms. Corresponding to a grill G on a topological space (X, τ), there exists a unique topology τ_G on X given by

$\tau_G = \{U \subset X \mid \Psi(X-U) = X-U\}$, where for any $A \subset X$, $\Psi(A) = A \cup \Phi(A) = \tau_G - cl(A)$. For any grill G on a topological space by (X, τ, G).

2.2. Definition A subset A of a topological space (X, τ) is called

1) a pre-open set (Mashhour *et al.,* 2009) if $A \subseteq$ int (cl(A)) and a pre-closed set if cl(int(A)) $\subseteq$ A.

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

3) an α-open set (Njastad, 1965) if $A \subseteq$ int(cl(int(A))) and an α - closed set (Maki *et al.,* 1993) if cl(int(cl(A))) $\subseteq$ A.

4) a semi-preopen set (Andrijevic, 1986) if $A \subseteq$ cl(int(cl(A))) and a semi-preclosed set (Arokiarani *et al.,* 1999) if (int(cl(A))) $\subseteq$ A.

2.3. Definition A subset A of a topological space (X, τ) is called

1) a generalized closed set (briefly g-closed) (Levine, 1970) if cl(A) $\subseteq$ U whenever $A \subseteq$ U and U is open in (X, τ).

2) a semi-generalized closed set (briefly sg-closed) (Bhattacharya and Lahiri, 1987) if scl(A) $\subseteq$ U whenever $A \subseteq$ U and U is semi-open in (X, τ).

3) a generalized semi-closed set (briefly gs-closed) (Arya and Nour, 1990) if scl(A) $\subseteq$ U whenever $A \subseteq$ U and U is open in (X, τ).

4) a generalized α-closed set (briefly gα-closed) (Maki *et al.,* 1993) if αcl(A) ⊆ U whenever A ⊆ U and U is α-open in (X, τ).

5) an α-generalized closed set (briefly α g-closed) (Maki *et al.,* 1994) if αcl(A) ⊆ U whenever A⊆ U and U is open in (X, τ).

6) a generalized semi-preclosed set (briefly gsp-closed) (Dontchev, 1995) if spcl(A) ⊆ U whenever A ⊆ U and U is open in (X, τ).

7) a generalized preclosed set (briefly gp-closed) (Maki *et al.,* 1996) if pcl(A) ⊆ U whenever A ⊆ U and U is open in (X, τ).

2.4. Definition A function f: (X, τ) → (Y, σ) is called

1) g-continuous (Balachandran *et al.,* 1991) if f^{-1}(V) is g-closed in (X, τ) for every closed set V in (Y, σ),
2) gp-continuous (Arokiarani *et al.,* 1999) if f^{-1}(V) is gp-closed in (X, τ) for every closed set V in (Y, σ),
3) gsp-continuous (Dontchev, 1995) if f^{-1}(V) is gsp-closed in (X, τ) for every closed set V in (Y, σ),
4) gα-continuous (Mashhour *et al.,* 1982) if f^{-1}(V) is gα-closed in (X, τ) for every closed set V in (Y, σ),
5) gs-continuous (Sundaram *et al.,* 1992) if f^{-1}(V) is gs-closed in (X, τ) for every closed set V in (Y, σ),
6) αg-continuous (Mashhour *et al.,* 1982) if f^{-1}(V) is αg-closed in (X, τ) for every closed set V in (Y, σ),

2.4. Theorem. (Arya and Nour, 1990) 1) If G_1 and G_2 are two grills on a space X with $G_1 \subset G_2$, then $\tau_{G1} \subset \tau_{G2.}$

2) If G is a grill on a space X and B ∉ G, then B is closed in (X, τ, G).

3) For any sunset A of a space X and any grill G on X, Φ(A) is τ_G-closed.

2.5. Theorem (Arya and Nour, 1990) Let (X, τ) be a topological space and G be any grill on X. Then

1) A ⊆ B (⊆ X) ⇒ Φ(A) ⊆ Φ(B);
2) A ⊆ X and A ∉ G ⇒ Φ (A) = ϕ;
3) Φ(Φ(A)) ⊆ Φ(A) = cl(Φ(A)) ⊆ cl(A), for any A ⊆ X;
4) Φ(A ∪ B) = Φ(A) ∪ Φ(B) for any A, B ⊆ X;
5) A ⊆ Φ(A) ⇒ cl(A) = τ_G – cl(A) = cl(Φ(A)) = Φ(A);
6) U ∈ τ and τ \ {ϕ} ⊆ G ⇒ U ⊆ Φ (U);
7) If U ∈ τ then U ∩ Φ(A) = U ∩ Φ(U∩A), for any A ⊆ X.

2.6. Theorem Let (X,τ) be a topological space and G be any grill on X. Then, for any A, B ⊆ X.

1) A ⊆ Ψ(A) (Arya and Nour, 1990);
2) Ψ(ϕ) = ϕ (Arya and Nour, 1990);
3) Ψ (A∪B) = Ψ(A) ∪ Ψ(B) (Arya and Nour, 1990);
4) Ψ (Ψ(A)) = Ψ(A) (Arya and Nour, 1990);
5) Int (A) ⊂ int(Ψ(A));
6) Int(Ψ(A ∩ B)) ⊂ Int (Ψ(A));
7) Int(Ψ(A ∩ B)) ⊂ Int (Ψ(B));
8) Int (Ψ(A)) ⊂ Ψ(A);
9) A ⊆ B ⇒ Ψ(A) ⊆ Ψ(B).

3. g - G – CONTINUOUS FUNCTIONS

3.1. Definition A subset A of a topological space (X, τ, G) is called a generalized grill closed

(briefly g - G - closed) set if Ψ (A) ⊆ U whenever A ⊆ U and U is open in X.

3.2. Definition A function f: (X, τ, G) → (Y, σ) is said to be g-G-continuous, if the inverse

image of every open set in (Y, σ) is g-G-open in (X, τ, G).

3.3. Definition A function f: (X, τ, G) → (Y, σ, H) is said to be g-G-irresolute, if f^{-1}(A) is

g-G-open in (X, τ, G) for every g-H-open set in (Y, σ, H).

3.4. Theorem Every g-continuous function is g-G-continuous but not conversely.

Proof. Let f: (X, τ, G) → (Y, σ) be an g-continuous. Let V be any open set in (Y, σ). Then f^{-1}(V) is g-open in (X, τ, G). Since every g closed set is g – G – closed set, f^{-1}(V) is g-G-open in (X, τ, G).Therefore is g-G-continuous.

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

{a,b}, X}. Define the function f: (X, τ, G) → (Y, σ) by f(a) = b, f(b) = a, f(c) = c. Then f is g-G-continuous but not g-continuous. Since for the g-G-open set V = {a} in (Y, σ), f^{-1}(V) is g-G-closed but not g-closed in (X, τ, G).

3.6. Theorem Every gs-continuous function is g-G-continuous but not conversely.

Proof. Let f: (X, τ, G) → (Y, σ) be an g-continuous. Let V be any open set in (Y, σ). Then $f^{-1}(V)$ is gs-open in (X, τ, G). Since every gs closed set is g – G – closed set, $f^{-1}(V)$ is g-G-open in (X, τ, G).Therefore is g-G-continuous.

3.7. Example Let X = {a,b,c}, τ = {ϕ, {a}, {a,b}, {a.c}, X} , σ ={ϕ, {a}, {a,c} X} and G =

{{b}, {a,b},X} Define the function f: (X, τ, G) → (Y, σ) by f(a) = a, f(b) = c, f(c) = b. Then f is g-G-continuous but not gs-continuous. Since for the g-G-open set V = {a} in (Y, σ), $f^{-1}(V)$ is g-G closed but not gs-closed in (X, τ, G).

3.8. Theorem Every sg-continuous function is g-G-continuous but not conversely.

Proof. Let f: (X, τ, G) → (Y, σ) be an g-continuous. Let V be any open set in (Y, σ). Then $f^{-1}(V)$ is sg-open in (X, τ, G). Since every sg closed set is g – G – closed set, $f^{-1}(V)$ is g-G-open in (X, τ, G).Therefore is g-G-continuous.

3.9. Example Let X = {a,b,c}, τ = σ = {ϕ, {a}, {a,b}, X} and G = { {b},{b,c}, X}. Define the

function f: (X, τ, G) → (Y, σ) by f(a) = a, f(b) = b, f(c) = c. Then f is g-G-continuous but not g-continuous. Since for the g-G-open set V = {a} in (Y, σ), $f^{-1}(V)$ is g-G closed but not sg-closed in (X, τ, G).

3.10. Theorem Every αg-continuous function is g-G-continuous but not conversely.

Proof. Let f: (X, τ, G) → (Y, σ) be an g-continuous. Let V be any open set in (Y, σ). Then $f^{-1}(V)$ is αg -open in (X, τ, G). Since every αg-closed set is g – G – closed set, $f^{-1}(V)$ is g-G-open in (X, τ, G).Therefore is g-G-continuous.

3.11. Example Let X = {a,b,c}, τ = {ϕ, {a}, {a,b}, {a,c}, X}, σ ={ϕ, {b}, {b,c} X} and G =

{{b}, {a,b}, X}. Define the function f: (X, τ, G) → (Y, σ) by f(a) = b, f(b) = c, f(c) = a. Then f is g-G-continuous but not αg -continuous. Since for the g-G-open set V = {b} in (Y, σ), $f^{-1}(V)$ is g- G closed but not αg -closed in (X, τ, G).

3.12. Theorem Every gα-continuous function is g-G-continuous but not conversely.

Proof. Let f: (X, τ, G) → (Y, σ) be an g-continuous. Let V be any open set in (Y, σ). Then $f^{-1}(V)$ is gα -open in (X, τ, G). Since every gα-closed set is g – G – closed set, $f^{-1}(V)$ is g-G-open in (X, τ, G).Therefore is g-G-continuous.

3.13. Example Let X = {a,b,c}, τ = σ = {{b}, X} and G = {{a}, {a,b}, X}. Define the function f: (X, τ, G) → (Y, σ) by f(a) = c, f(b) = a, f(c) = a. Then f is g-G-continuous but not gα-continuous. Since for the g-G-open set V = {b} in (Y, σ), $f^{-1}(V)$ is g- G closed but not gα -closed in (X, τ, G).

3.14. Theorem Every gp-continuous function is g-G-continuous but not conversely.

Proof. Let f: (X, τ, G) → (Y, σ) be an g-continuous. Let V be any open set in (Y, σ). Then $f^{-1}(V)$ is gp -open in (X, τ, G). Since every gp-closed set is g – G – closed set, $f^{-1}(V)$ is g-G-open in (X, τ, G).Therefore is g-G-continuous.

3.15. Example Let X = {a,b,c}, τ = {ϕ, {a}, {b}, {a,b}, X}, σ = {ϕ, {a}, {a,b}, X} and G =

{{a}, {a,c}, X}. Define the function f: (X, τ, G) → (Y, σ) by f(a) = b, f(b) = a, f(c) = c. Then f is g-G-continuous but not gp-continuous. Since for the g-G-open set V = {a} in (Y, σ), $f^{-1}(V)$ is g- G closed but not gp -closed in (X, τ, G).

3.16. Theorem Every gsp-continuous function is g-G-continuous but not conversely.

Proof. Let f: (X, τ, G) → (Y, σ) be an g-continuous. Let V be any open set in (Y, σ). Then $f^{-1}(V)$ is gsp -open in (X, τ, G). Since every gsp-closed set is g – G – closed set, $f^{-1}(V)$ is g-G-open in (X, τ, G).Therefore is g-G-continuous.

3.17. Example Let X = {a,b,c}, τ = {ϕ, {a}, {a,b}, {a,c}, X}, σ = {ϕ, {a}, {a,b}, X} and G =

{{a}, {a,b}, X}. Define the function f: (X, τ, G) → (Y, σ) by f(a) = a, f(b) = c, f(c) = b. Then f is g-G-continuous but not gsp-continuous. Since for the g-G-open set V = {a} in (Y, σ), $f^{-1}(V)$ is g- G closed but not gsp -closed in (X, τ, G).

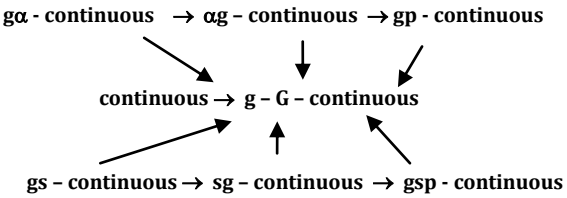
3.18.Theorem Let f: (X, τ, G) → (Y, σ) is g-G-continuous and g: (Y, τ) → (Z, η) is continuous then g o f : (X, τ, G) → (Z, η) is g-G-continuous.

Proof. Let g be a continuous function and V be any open in (Z, η), then $f^{-1}(V)$ is open in (Y, σ). Since f is g-G-continuous, $f^{-1}(g^{-1}(V)) = (g \circ f)^{-1}(V)$ is g-G-open in (X, τ, G).Hence g o f is g-G-continuous.

3.19. Theorem Let f: (X, τ, G) → (Y, σ, H) and g: (Y, τ, H) → (Z, η, L) are g-G-irresolute then g o f : (X, τ, G) → (Z, η, L) is g-G-irresolute.

Proof. Let g be a g-G-irresolute and V be any g-L-open in (Z, η, L), then $f^{-1}(V)$ is g-G-open in (Y, σ, H). Since f is g-G-irresolute, $f^{-1}(g^{-1}(V)) = (g \circ f)^{-1}(V)$ is g-G-irresolute in (X, τ, G).Hence g o f is g-G-irresolute.

3.20. Remarks



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B-CHROMATIC NUMBER OF CENTRAL GRAPH OF LADDER GRAPH AND COMPLETE GRAPH

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ABSTRACT

In this paper, we discuss about the b-colouring andb-chromatic number of Central graph of Ladder graph and Central graph of Complete graph denoted as $C(L_n)$ and $C(K_n)$ respectively. Also we discuss about the structural properties of $C(L_n)$ and $C(K_n)$.

Keywords: Central graph, b-colouringand b-chromatic number.

1. INTRODUCTION AND PRELIMINARIES

All graphs considered here are finite and simple. Notations and terminology not defined here will conform to those in (Bondy and Murty, 1976). For a graph G, let $V(G)$, $E(G)$, $p(G)$, $q(G)$ and $\Delta(G)$,$\delta(G)$, respectively, be the set of vertices, the set of edges, the order, the size, the maximum and minimum degree of G.

Let G be a graph without loops and multiple edges with vertex set $V(G)$ and edge set $E(G)$. The smallest number k for which G admits a colouring with k colours is the chromatic number $\chi(G)$ of G. Many graph invariants related to colourings have been defined. Most of them try to minimize the number of colours used to colour the vertices under some constraints. For some invariants, it is meaningful to try to maximize this number. The b-chromatic number is one such example.

A *b-colouring* (Jakovac and Klavzar, 2010; Jakovac and Peterin, 2012; Kouider, 2002; Kouider and Zaker, 2006) of a graph G is a proper colouring of the vertices of G such that there exist a vertex in each colour class joined to at least a vertex in each other colour class; such a vertex is called a dominating vertex. The *b-chromatic number* of a graph G, denoted by $\varphi(G)$, is the maximal integer k such that G may have a b-colouring by k colours. This parameter has been derived by Irving and Manlove in the year 1999.

The central graph (Thilagavathi *et al.,* 2010; VernoldVivin *et al.,* 2008; Vijayalakshmi and Thilagavathi, 2010) of any graph G is obtained by subdividing each edge of G exactly once and joining all the non adjacentvertices of G. By the definition pC(G) = p + q. For any (p, q) graph there exactly p vertices of degree p -1 and q vertices of degree 2 in C (G).

2. THE B-CHROMATIC NUMBER OF CENTRAL GRAPH OF LADDER GRAPH

2.1.Theorem

For any integer $1<n<20$, $\varphi[C(L_n)] = n+\left\lceil\frac{n}{2}\right\rceil$

Proof

Let $L_n$be any ladder with vertices [12] $v_1,v_2...v_n$ labeled in anticlockwise direction. Let v_{ij} be the newly introduced vertex in the edge connecting v_i and v_j, $1<i, j< 2n$ in $C(L_n)$. Now in $C(L_n)$, we see that each v_i is adjacent with all the vertices except v_{i+1} and $v_{2n-(i-1)}$ for $i=1,2,3...2n$. Let $S=\{v_{ij}/1<i,j< 2n\}$.

Now assign a proper colouring to these vertices as follows. Consider a colour class $C=\{c_1,c_2,c_3..c_n\}$.For $i=1,2,3...2n$ assign the colour c_i to the vertex v_i. Due to the above mentioned non adjacency this will not produce a b-chromatic colouring.

To overcome this, assign a proper colouring to v_{ij}'s. consider an arbitrary vertex v_i, but v_i is not adjacent with v_{i+1} and v_{i-1},thus the vertex v_i to realize the colourc_i, we should colour$v_{i,i+1}$as c_{i-1} and $v_{i,i-1}$ as c_{i+1}. Now v_i will realize the colour c_i. Next consider the vertex v_{i+1} which is coloured as c_{i+1}. In order to realize the colour $c_{i+1,}$ colour the two neighbors of v_{i+1} as c_{i+1} and c_i but by previous colouring v_i had left out only one vertex to be coloured. Thus realization of v_{i+1} is not possible. Proceeding in the same manner this will not be possible for remaining vertices. This implies that assigning different colours to v_i is not possible otherwise there should be repetition of colours. A close examination will reveal that there should be minimum of $\left\lceil\frac{n}{2}\right\rceil$ repetitions.

Now assign the colour $c_{i-[i/6]}$ to the vertex v_i for $i=1,2,3,...2n-1$ and assign the colour $c_{i-\{[i/6]+1\}}$ to the vertex v_{2n}.

To make the above colouring as b-chromatic, assign a proper colouring to the remaining $v_{ij}'s$. Suppose if we assign any new colour to any of the $v_{ij}'s$ as $c_{i-\{[i/6]+1\}}$ it contradicts the definition of b-chromatic colouring. Hence we should assign only the existing colours to $v_{ij}'s$ inorder that all the vertices $v_1, v_2 \ldots v_{2n}$ realizes its own colour. Thus by the colouring procedure and under observation the above said colouring is maximal and b-chromatic colouring.

Example

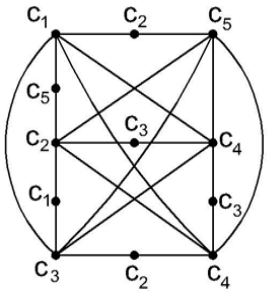

Figure:1 $\varphi[C(L_3)] = 5$

2.2. Structural Properties of Central Graph of Ladder Graph

- Number of vertices in $C(L_n) = 5n-2$
- Maximum degree in $C(L_n)$ i.e. $\Delta = 2n-1$
- Minimum degree in $C(L_n)$ i.e. $\delta = 2$

3. B-CHROMATIC NUMBER OF CENTRAL GRAPH OF COMPLETE GRAPH

3.1. Theorem

For any integer $n>3$, $\varphi[C(K_n)] = n-1$

Example

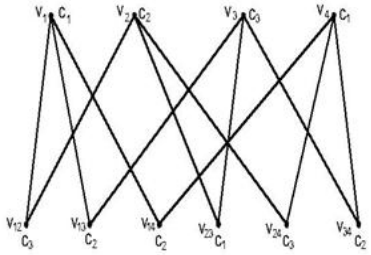

Figure :2 $\varphi[C(K_4)] = 3$

3.2. Structural Properties of Central graph of Complete Graph

- Number of vertices in $C(K_n) = \frac{n(n+1)}{2}$
- Number of edges in $C(K_n) = n(n-1)$
- Maximum degree in $C(K_n) = (n-1)$
- Minimum degree in $C(K_n) = 2$
- n vertices with maximum degree $n-1$ and $\frac{n(n-1)}{2}$ vertices of degree .

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SEMI $^{\#}$GENERALIZED α–CONTINUOUS FUNCTIONS

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ABSTRACT

In this paper we introduce and discuss some basic properties and preservation theorems of slightly s$^{\#}$gα-continuous functions.

Keywords: clopen, s$^{\#}$gα-open, slightly s$^{\#}$gα-continuity.

1. INTRODUCTION

The notion of s$^{\#}$gα-closed sets in a topological space was introduced by V. Kokilavani and M. Vivek Prabu, 2013. The concept of slightly continuous functions were introduced and investigated by R.C. Jain, 1980.

In this paper we introduce the notion of slightly s$^{\#}$gα-continuous functions and discuss their basic properties. Throughout this paper X, Y and Z 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

2.1. Definition A subset A of a topological space X is said to be

(i) g-closed (Levine, 1970) if cl(A) ⊆ U, whenever A ⊆ U and U is open. The complement of a g-closed set is said to be g-open.

(ii) g$^{\#}\alpha$-closed (Nano *et al.,* 2004) if αcl(A) ⊆ U, whenever A ⊆ U and U is g-open. The complement of a g$^{\#}\alpha$-closed set is said to be g$^{\#}\alpha$-open.

(iii) $^{\#}$gα-closed (Devi *et al.,* 2009) if αcl(A) ⊆ U, whenever A ⊆ U and U is g$^{\#}\alpha$-open. The complement of a $^{\#}$gα-closed set is said to be $^{\#}$gα-open.

(iv) s$^{\#}$gα-closed (Vivek Prabu and Kokilavani, 2013) if scl(A) ⊆ U, whenever A ⊆ U and U is $^{\#}$gα-open. The complement of a s$^{\#}$gα-closed set is said to be s$^{\#}$gα-open.

2.2. Definition A function f : X ⟶ Y is s$^{\#}$gα-continuous (Vivek Prabu and Kokilavani, 2013) if f^{-1}(V) is s$^{\#}$gα-closed in X for every closed set V in Y.

2.3. Definition A function f : X ⟶ Y is slightly continuous (Jain, 1980) if f^{-1}(V) is open in X for every clopen set V in Y.

3. SLIGHTLY s$^{\#}$gα-CONTINUOUS FUNCTIONS

3.1. Definition A function f : X ⟶ Y is said to be slightly s$^{\#}$gα-continuous if f^{-1}(V) is s$^{\#}$gα-open in X for every clopen set V in Y.

3.2. Theorem For a function f : X ⟶ Y, the following are equivalent:

(a) f is slightly s$^{\#}$gα-continuous.

(b) f^{-1}(V) ∈ s$^{\#}$gαO (X) for each V ∈ CO (Y).

(c) f^{-1}(V) is s$^{\#}$gα-clopen for each V ∈ CO (Y).

Proof: (a) ⟹ (b): Let V ∈ CO (Y) and let x ∈ f^{-1}(V). Then f(x) ∈ V. Since f is slightly s$^{\#}$gα-continuous, there is a U ∈ s$^{\#}$gαO (X, x) such that f(U) ⊂ V. Thus f^{-1}(U) = $\cup_x${U: x ∈ f^{-1}(V)}, that is f^{-1}(U) is a union of s$^{\#}$gα-open sets. Hence f^{-1}(U) ∈ s$^{\#}$gαO (X).

(b) ⟹ (c): Let V ∈ CO (Y). Then (Y – V) ∈ CO (X). By hypothesis f^{-1}(Y – V) = X – f^{-1}(V) ∈ s$^{\#}$gαO (X). Thus f^{-1}(V) is s$^{\#}$gα-closed.

(c) ⟹ (a): The proof is obvious.

3.3. Theorem If f : X ⟶ Y is slightly s$^{\#}$gα-continuous and g : Y ⟶ Z is slightly s$^{\#}$gα-continuous, then their composition g∘f is slightly s$^{\#}$gα-continuous.

Proof: Let V ∈ CO (Z), then g^{-1}(V) ∈ CO (Y). Since f is slightly s$^{\#}$gα-continuous, $f^{-1}(g^{-1}(V)) = (g \circ f)^{-1}(V)$ ∈ s$^{\#}$gαO (X). Thus g∘f is slightly s$^{\#}$gα-continuous.

3.4. Theorem The following are equivalent for a function f : X ⟶ Y:

(a) f is slightly s$^{\#}$gα-continuous.

(b) for each x ∈ X and for each V ∈ CO (Y, f(x)), there exists a s$^{\#}$gα-clopen set U such that f(U) ⊂ U.

(c) for each closed set F of Y, f^{-1}(F) is s$^{\#}$gα-closed.

(d) f(cl(A)) ⊂ s$^{\#}$gαcl(f(A)) for each A ⊂ X.

(e) cl(f^{-1}(B)) for each B ⊂ Y.

Proof: (a) ⟹ (b): Let x ∈ X and V ∈ CO (Y, f(x)), by theorem 3.2 $f^{-1}(V)$ is clopen. Put U = $f^{-1}(V)$, then x ∈ U and f(U) ⊂ V.

(b) ⟹ (c): It is obvious.

(c) ⟹ (d): Since $s^{\#}g\alpha cl(f(A))$ is the smallest $s^{\#}g\alpha$-closed set containing f(A), hence by (c), we have (d).

(d) ⟹ (e): For each B ⊂ Y, $f(cl(f^{-1}(B))) \subset s^{\#}g\alpha cl(f(f^{-1}(B))) \subset s^{\#}g\alpha cl(f(B))$. Hence $f(cl(f^{-1}(B))) \subset f^{-1}(s^{\#}g\alpha cl(f(B))) \Longrightarrow cl(f^{-1}(B)) \subset s^{\#}g\alpha cl(f(B))$.

(e) ⟹ (a): Let V ∈ CO (Y). Then (Y – V) ∈ CO (X), by (e), we have $cl(f^{-1}(Y - V)) \subset f^{-1}(s^{\#}g\alpha cl(Y - V)) = f^{-1}(Y - V)$, since every closed set is $s^{\#}g\alpha$-closed, thus $f^{-1}(Y - V) = X - f^{-1}(V)$ is closed and thus $s^{\#}g\alpha$-closed. Hence $f^{-1}(V) \in s^{\#}g\alpha O(X)$ and f is slightly $s^{\#}g\alpha$-continuous.

3.5. Definition A function f : X ⟶ Y is called almost contra $s^{\#}g\alpha$-continuous if $f^{-1}(V)$ is $s^{\#}g\alpha$-closed in X for every regular open set V in Y.

3.6. Theorem The following are equivalent for a function f : X ⟶ Y:

(a) f is almost contra $s^{\#}g\alpha$-continuous.

(b) $f^{-1}(F) \in s^{\#}g\alpha C(X)$ for every F ∈ RO (Y).

(c) for each x ∈ X and for each regular closed subset F in Y containing f(x), there exists a $s^{\#}g\alpha$-closed set U in X containing x such that f(U) ⊆ F.

(d) for each x ∈ X and for each regular closed subset V in Y not containing f(x), there exists a $s^{\#}g\alpha$-open set K in X not containing x such that $f^{-1}(V) \subseteq K$.

(e) $f^{-1}(int(cl(G))) \in s^{\#}g\alpha C(X)$ for every open subset G of Y.

(f) $f^{-1}(cl(int(F))) \in s^{\#}g\alpha O(X)$ for every closed subset F of Y.

Proof: (a) ⟹ (b): Let F ∈ RO (Y). Then Y – F ∈ RC (Y). By (a), $f^{-1}(Y - F) = X - f^{-1}(F) \in s^{\#}g\alpha O(X)$. Hence $f^{-1}(F) \in s^{\#}g\alpha C(X)$.

(b) ⟹ (a): The Proof is similar.

(b) ⟹ (c): Let F be any regular open set in Y containing f(x). By (b), $f^{-1}(F) \in s^{\#}g\alpha C(X)$ and x ∈ $f^{-1}(F)$. Take U = $f^{-1}(F)$. Then f(U) ⊆ F.

(c) ⟹ (b): Let F be any regular open set in Y and x ∈ $f^{-1}(F)$. From (c), there exists a $s^{\#}g\alpha$-closed set U in X containing x such that f(U) ⊆ F. We have $f^{-1}(F)$ = U. Thus $f^{-1}(F)$ is $s^{\#}g\alpha$-closed.

(c) ⟹ (d): Let V be any regular closed set in Y not containing f(x). Then Y – V is a regular open set containing f(x). By (c), there exists a $s^{\#}g\alpha$-closed set U in X containing x such that f(U) ⊆ Y – V. Hence $U \subseteq f^{-1}(Y - V) \subseteq X - f^{-1}(V)$ and then $f^{-1}(V) \subseteq X - U$.Take H = X – U, we obtain that H is a $s^{\#}g\alpha$-open set in X not containing x.

(d) ⟹ (c): The Proof is similar.

(b) ⟹ (e): Let G be an open subset of Y. Since int(cl(G)) is regular open, then by (b), it follows that $f^{-1}(int(cl(G))) \in s^{\#}g\alpha C(X)$.

(e) ⟹ (b): The Proof is similar.

(a) ⟹ (f): Let F be a closed subset of Y. Since cl(int(G)) is regular closed, then by $f^{-1}(F) \in s^{\#}g\alpha C(X)$, it follows that $f^{-1}(cl(int(F))) \in s^{\#}g\alpha O(X)$.

(f) ⟹ (a): The Proof is similar.

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A SIMPLE, EFFICIENT, ONE-POT THREE-COMPONENT DOMINO SYNTHESIS OF *HANTZSCH* 1,4-DIHYDROPYRIDINE UNDER MILD CONDITIONS

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ABSTRACT

A series of substituted *Hantzsch* 1, 4-Dihydropyridine derivatives were synthesized and the structures of these compounds were established on the basis of analytical and spectral data such as FT-IR and ^{1}H-NMR. The advantages of this system are one-step procedure, high yields of the products and the ability to carry out large-scale reactions.

Keywords: Hantzsch reaction, 1,4-dihydropyridines, cyclization, one-pot procedure.

1. INTRODUCTION

Multicomponent reactions (MCRs) are one-pot processes that combine three or more substrates simultaneously (Guillena, *et al.,* 2007). Such processes are of great interest in diversity-oriented synthesis, especially to generate compound libraries for screening purposes.

Dihydropyridine (DHP) is a molecule based up on pyridine, and the parent of a class of molecules that have been semi-saturated with two substituent's replacing one double bond. They are particularly well known in pharmacology as L-type calcium channel blockers, used in the treatment of hypertension compared with certain other L-type calcium channel blockers [For example those of the phenyl alkylamine class such as verpamil] which have significant action at the heart, they are relatively vascular selective action at the heart, they are relatively vascular selective in their mechanism of action in lowering blood pressure.

Some of the representative compounds of this class possess acaricidal, insecticidal, bactericidal and herbicidal activities (Kawase *et al.,* 2002). It has been recognized as vital drugs in the treatment of angina and hypertension (Janis and Triggle, 1983; Boecker and Guengerich, 1986). Some of them have been commercialized and it has been proven that their therapeutic success is related to their efficacy to bind to calcium channels and consequently to decrease the passage of the trans membrane calcium current, associated in smooth muscle with a long lasting relaxation and in cardiac muscle with a reduction of contractility throughout the heart (Bossert *et al.,* 1981; Love *et al.,* 1974).

In addition DHP finds applications in stereo specific hydrogen transfer reactions. Krechi and Smrckova have reported stereo-specific reduction of phenyl glyoxylic and pyruvic acid using DHP to biomimetic models of lactase dehydrogenase. Recently, dihydropyridines are used as organo catalysts for asymmetric reactions such as hydrogenation of quinolines in the synthesis of alkaloids (Rueping *et al.,* 2006), asymmetric reductive amination of aldehydes (Hoffmann *et al.,* 2006) and hydrogenation of α,β,unsaturated aldehydes and ketones, (Martin and List, 2006), recent studies suggest several other medicinal applications including neurotropic, antidiabetic, membrane protecting, as well as anticancer, antibacterial, and antiviral activities.

Development of an efficient and versatile method for the preparation of 1,4-dihydropyridines is an active ongoing research area (Bocker and Guengerich, 1986; Breitenbucher and Figliozzi 2000; Gordeev *et al.,* 1996; Vanden Eynde and Mayence, 2000) and there is scope for further improvement toward synthesis of new derivatives of 1,4-dihydropyridines with milder reaction conditions and improved yields. Therefore, we decided to synthesize new derivatives of 1,4-dihydropyridines and provide a clean and easy work-up.

2. EXPERIMENTAL

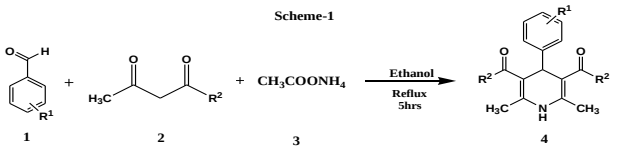
All chemicals were purchased from Sigma-Aldrich, India. The reactions were monitored by TLC. The products were isolated and identified by comparison of their physical and spectral data. IR spectra of the products were recorded on shimadzu spectrometer in the range 500- 4000cm^{-1}. ^{1}H NMR spectra were recorded using $CDCl_3$ as solvent chemical shifts were expressed in 'δ'units (ppm) and quoted downfield from TMS as internal standard by the instrument :*Bruker Avance III*

3. SYNTHESIS OF HANTZSCH 1,4-DIHYROPYRIDINE:

A mixture of substituted benzaldehyde (1) (1mmol), β-dicarbonyl (2) (2mmol), ammonium acetate (3)

(3mmol) and ethanol (10ml) were successively changed in to 100ml round bottom flask. Then the reaction mixture was heated in a water bath for 5 hours at 100ºC and a yellow colour product was gradually formed (scheme-1). The completion of reaction is tested by thin layer chromatography [TLC] the resulting product (4a-4l) was recrystallized with ether.

Scheme-1



1 2 3 4

4. RESULTS AND DISCUSSION:

Various 1,4-dihydropyridine derivatives were synthesized. In all the cases, the desired protect were obtained in high yields. Mechanistically, this reaction is a complex reaction as it involves three reactants at differing stoichiometry. There could be two reaction pathways to obtain 1,4-dihydropyridine: (1) The reaction may go through aldol condensation of β-dicarbonyl with benzaldehyde and subsequent reaction of aldol with enamine (obtained through condensation of one molecule of β-dicarbonyl with NH_4OAc) or (2) The reaction may go through condensation of two molecules of β-dicarbonyl with NH_4OAc forming an imine which subsequently undergoes condensation with benzaldehyde to form 1,4-dihydropyridine. Both electro-rich and electron-deficient aromatic aldehydes worked well. Many of the pharmacologically significant substitution patterns can be introduced with good efficiency. (Table-1)

4.1.Spectral data for selected products:

3,5-diethyl-2,6-dimethyl-4-(2,3-dichlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. (4d) IR υ (cm^{-1}) = 3332.14, 1732.15, 1566, 1098.51,730,648.11; ^1H NMR ($CDCl_3$) δ (ppm) = 1.20-1.16 (t,6H), 2.41(s,6H),4.10-4.04(q,4H),4.95(s,1H),5.64(s,1H),7.31-7.22(m,3H)

3,5-diethyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.(4e) IR υ (cm^{-1}) = 3336.9, 1727.3, 1645.35, 1548.9, 1207.49, 1122.62, 733.95; ^1H NMR ($CDCl_3$) δ (ppm) = 1.23-1.19 (t,6H), 2.32(s,6H),4.10-4.06(q,4H),4.98(s,1H),5.64(s,1H),7.28-7.09(m,4H)

2,3,5,6-tetramethyl-4-(4-methoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylate.(4l)

IR υ (cm^{-1}) = 3348.57, 1693.57, 1493.93, 1436.07, 1218.10, 748.41, 680.90; ^1H NMR ($CDCl_3$) δ (ppm) = 2.32(s,6H), 3.64(s,6H),3.74(s,3H),4.94(s,1H),5.62(s,1H),6.73-7.18(m,4H)

5. CONCLUSION

In conclusion, we have developed a simple and efficient synthetic protocol for the synthesis of a wide variety of Hantzsch 1,4-dihydropyridine derivatives. Mild reaction conditions, cost efficiency, simplicity in operation, and large-scale applicability are some significant features of this protocol. There is a wide scope to develop enamine chemistry from these molecules with electron deficient systems or cyclization. This can lead to excellent new molecules for further biological evaluations.

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Table-1-Analytical data for 1,4-dihydropyridine derivatives

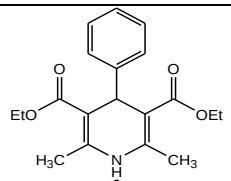
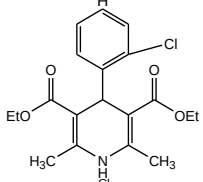
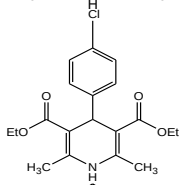
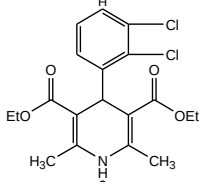
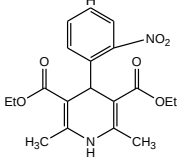
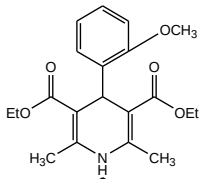
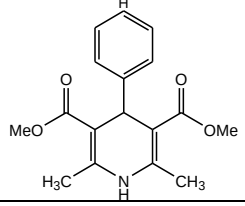
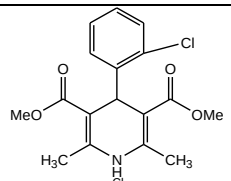
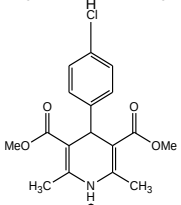
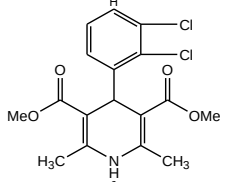
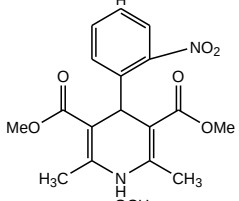
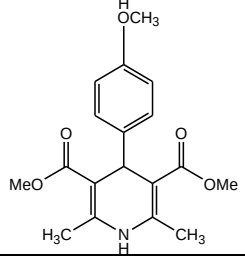
Entry Products	R^1	R^2	Products	Yield[a] (%)	Melting point (ºC)
4a	C_6H_5	OEt		79	175
4b	2-Cl-C_6H_4	OEt		74	143
4c	4-Cl- C_6H_4	OEt		79	160
4d	2,3-di-Cl-C_6H_4	OEt		78	145
4e	2-NO_2-C_6H_4	OEt		74	174
4f	2-OMe- C_6H_4	OEt		75	168
4g	C_6H_5	OMe		79	165

Table 1 Analytical data for 1,4-dihydropyridine derivatives (Continued)

Entry Products	R^1	R^2	Products	Yield[a] (%)	Melting point (ºC)
4h	2-Cl-C_6H_4	OMe		78	168
4i	4-Cl- C_6H_4	OMe		72	150
4j	2,3-di-Cl-C_6H_4	OMe		79	172
4k	2-NO_2-C_6H_4	OMe		78	124
4l	4-OMe- C_6H_4	OMe		75	180

a- Isolated yields.

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ANTIMICROBIAL ACTIVITY OF COPPER(II) COMPLEX OF 1-(1*H*-BENZIMIDAZOL-2-YL)-*N*-(TETRAHYDROFURAN-2-YLMETHYL)METHANAMINE

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ABSTRACT

A mononuclear copper(II) complex ([Cu(L)(Cl)]Cl where L is 1-(1*H*-benzimidazol-2-yl)-*N*-(tetrahydrofuran-2-ylmethyl)methanamine) was synthesized. The synthesized complex was characterized using various physicochemical techniques like cyclic voltammetry and elemental analysis, ESI-MS, UV–Visible, Infra red and EPR. The antimicrobial activities of the ligand and their metal complexes were screened by disc diffusion method and found that the metal complexes have higher antimicrobial activity than the free ligand.

Keywords: antimicrobial, copper, benzimidazol.

1. INTRODUCTION

Copper is a biologically relevant element and many enzymes that depend on copper for their activity have been identified (Barton, 1986; Dervan, 1986; Dhar and Chakravarty, 2003; Garcia-Raso, *et al.,* 2003), Copper(II) is a substitutionally labile metal ion. So multidentate ligands are believed to be better than bidentate ligands in keeping the copper(II) ion chelated in solution. Typically, upon association with dioxygen or hydrogen peroxide these copper complexes are thought to perform reactive intermediates. Sigman etal have shown that the bis(phen) copper complex acts as an efficient nuclease by oxidative cleavage mechanism in the presence of molecular oxygen and a reducing agent (Hemmert, *et al.,* 2001; Navarro, 2003; Sreedhara *et al.,* 2000). Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause disease. So more interests have been shown in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cells. The biological studies of metal complexes highlighted the potential of antioxidant activity of copper(II) complex with bioactive ligand. The present work stems from our interest to design copper(II) complex with tetrahydro furyl amine based ligand. We have synthesized a copper complex[Cu(L1)(Cl)]Cl where L1 is tetrahydro furyl amine based unsymmetrical tridentate ligand.

2. MATERIALS AND METHODS

1-(tetrahydrofuran-2-yl) methanamine, was procured from Sigma Aldrich, USA and used as received. Other materials like sodium borohydride and solvents like methanol, acetonitrile and dichloromethane were of reagent grade. Benzimidazole carbaldehyde was prepared using published procedure.] UV–visible spectrum of the complex was recorded on a Perkin–Elmer Lambda 35 double beam spectrophotometer at 25°C. Electron paramagnetic resonance spectrum of the copper(II) complexes were obtained on a Varian E 112 EPR spectrometer. IR spectrum was recorded as KBr pellets in the 400-4000 cm^{-1} region using a Shimadzu FT–IR 8000 spectrophotometer. Positive ion electrospray ionization mass spectrum of the complex was obtained by using Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer.

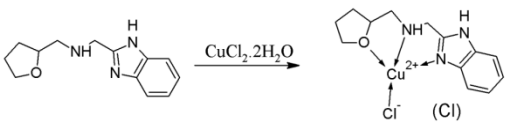

Figure 1. Structure of Copper Complex

2.1 Synthesis of 1-(1H-benzimidazol-2-yl)-N-(tetrahydrofuran-2-ylmethyl)methanamine (L)

Benzimidazole-2-aldehyde (0.767 g, 5 mmol) and tetrahydrofurfuryl amine (0.505 g, 5 mmol) were mixed in methanol (20 mL) and stirred well for one day. Sodium borohydride (0.28 g, 7.5 mmol) was added to the above solution at 0°C and the reaction mixture was stirred overnight at room temperature. The reaction mixture was

rotaevaporated to dryness and the residue was dissolved in water (15 mL) and extracted with dichloromethane. The organic layer was dried and the solvent was evaporated to give the ligand as brown oil, which was used as such for the preparation of complex. Yield: 1.016 g (88 %).

2.2 Synthesis of [Cu (L) (Cl)] Cl (1)

The complex was prepared in good yield from the reaction of $CuCl_2 \cdot 2H_2O$ in methanol with L. The ligand, L (0.68 g, 3 mmol) and $CuCl_2 \cdot 2H_2O$ (0.5 g, 3 mmol) were dissolved in methanol individually and the solutions were warmed. To the hot solution of L5, copper chloride was added slowly and stirred for 3 hours. The resulting solution was cooled to room temperature and the green colored copper–L complex separated out was filtered and dried. Yield: 0.921 g (84 %). Anal. Calc. for $C_{13}H_{17}Cl_2CuN_3O$: C, 42.69; H, 4.68; N, 11.49; Cu, 17.37; Found: C, 42.67; H, 4.62; N, 11.43; Cu, 17.31 %. FT-IR (KBr pellet) cm^{-1}: 3302, 3067, 1624, 1589, 1093, 748, 621. UV (nm): 277, 365, 682. ESI-MS: m/z = 365.27 $[M - Cl]^+$.

2.3 Antimicrobial Assay

2.3.1. Micro-organisms used

Five species of bacteria, two gram positive (*Streptococcus faecalis & Bacillus subtilis*) and three gram negative (*Escherichia coli, Klebsiella pneumonia* & *Salmonella paratyphi*) were obtained from KMCH, Coimbatore.

2.3.2. Preparation of Inoculum

A loopful of strain was inoculated in 30 mL of nutrient broth in a conical flask and incubated on a rotary shaker at 37°C for 24 hours to activate the strain.

2.3.3. Bioassay

The bioassay used was the standard Agar Disc Diffusion assay. Mueller Hinton Agar was prepared for the study. Mueller Hinton agar plates were swabbed with a suspension of each bacterial species, using a sterile cotton swab. Subsequently, the sterilized filter paper discs were completely saturated with the test compound. The impregnated dried discs were placed on the surface of each inoculated plate. The plates were incubated overnight at 37°C. Each compound was tested against each organism in triplicate. Methanol was used as negative control. Standard discs of Ampicillin served as positive antibacterial control. The test materials having antimicrobial activity inhibited the growth of the micro organisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in mm.

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization

Ligand L was synthesized by condensing tetrahydro furfuryl amine with benzimidazole aldehyde to form Schiff base followed by reduction with sodium borohydride. It was characterized by ESI-MS and ^1H NMR spectra. The copper(II) complex of the ligand was prepared by the reaction between copper(II) chloride and the corresponding ligand in equimolar quantities using methanol as solvent. The complex was obtained in good yield and characterized by using elemental analysis, UV-Vis, ESI-MS and EPR spectral techniques. The structure for the present complex is shown in figure 1.

The ESI mass spectra of [Cu(L)(Cl)]Cl displayed the molecular ion peak at m/z 367.27 which is reliable with the proposed molecular formula of the corresponding copper (II) complex. The electronic spectrum of the complex shows a low energy ligand field (LF) band (682 nm) and a high energy ligand based band (277 nm). Broad ligand field transition has been observed for all the four complexes in the region of 682 nm. Three d–d transitions are possible for copper (II) complexes. They are d_{xz},d_{yz}- $d_{x^2-y^2}$, d_{z^2}-$d_{x^2-y^2}$ and d_{xy}-$d_{x^2-y^2}$. However, only a single broad band is observed for the copper (II) complex. This indicates the total sum of all the above transitions. The broadness associated with the d–d bands is generally taken as an indication of the geometrical distortion of the complex from perfect planar symmetry.

IR spectra provide the valuable information about the nature of the binding mode and functional group attached to the metal ion. The peak observed at 1620 cm^{-1} have been assigned to the C=N stretching frequencies of benzimidazole group. IR peak observed in the region of 3248 cm^{-1} indicates the stretching vibration of NH group of ligand L.

The epr spectrum of complex shows axial signal at 300 K from a static copper (II) centre with dx^2-y^2 as the ground state. The g value is 2.07. The broad epr spectrum and its g value confirm the formation of the copper (II) complex. Also it confirms that the complex is paramagnetic.

The redox behavior of copper complex is studied with the help of cyclic voltammetry. Copper complex shows an irreversible peak at 0.51 V at a scan rate of 100 mVs^{-1}. The redox process is assigned to Cu^{II}/Cu^{I} couple (Mistra and Pandey, 1992).

3.2. Antimicrobial Activity

The *in vitro* biological screening effects of the investigated compounds were tested against the bacteria: *Salmonella paratyphi, Streptococcus faecalis, Escherichia coli, Klebsiella pneumonia* and *Bacillus subtilis* by the disc diffusion method. In the modified disc diffusion assay the majority of the complexes showed some activity in the screen. Results displayed in Table 1, clearly indicate that the inhibitions are much larger by metal complexes as compare to the metal free ligand.

The observed zone of inhibition order of present complex was *S. faecalis > B. subtilis > K. pneumonia > S. paratyphi > E. coli.* Interestingly the copper complex showed an efficient inhibitory activity against the bacterial pathogen *Streptococcus faecalis*.

The increased activity of the metal chelates can be explained on the basis of chelation theory. Also activity increases with concentration of the metal complexes. The chelation tends to make the ligands act as more powerful and potent bacterial agents, thus killing of more bacteria than the ligand. It is observed that in complexes the positive charge of the metal partially shared with the donor atoms present in the ligand and there may be π-electron delocalization over the whole chelate ring. Such an electron delocalization enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.

Table 1. Antimicrobial activity of copper complex

S. No	Bacteria	Control Ampicillin (mm)	Zone of inhibition (mm)	
			L5	12
1	*Streptococcus faecalis*	13.2±0.51	10.17±0.57	17.3±0.9
2	*Bacillus subtilis*	14.5±0.4	7.49±0.34	13.6±0.29
3	*Klebsiella pneumonia*	15±0.04	8.64±0.31	9±0.38
4	*Salmonella paratyphi*	15±0.57	6.71±0.23	8±0.13
5	*Escherichia coli*	16.3±0.15	5.44±0.05	6±0.26

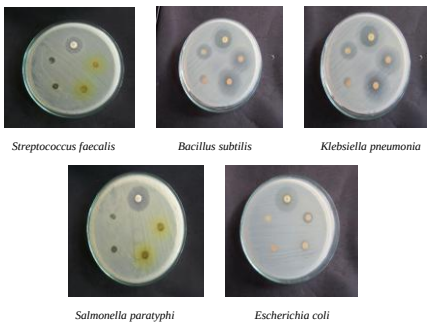

Figure 1. Antimicrobial activity of copper complex

4. CONCLUSION

In summary, we have synthesized and characterized a new mononuclear copper complex having tridentate reduced Schiff base. The antimicrobial activities of the ligand and their metal complexes were screened by disc diffusion method and found that the metal complexes have higher antimicrobial activity than the free ligand.

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SCREENING OF PHYTOCHEMICAL CONSTITUENTS IN SOLVENT EXTRACTS OF *ACORUS CALAMUS*

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ABSTRACT

Plants have the ability to synthesize mixtures of structurally diverse bio-active compound, with multiple and mutually potential therapeutic effects. *Acorus calamus* a medicinal harp is known to be rich in β asarone in its composition. The rhizome of this plant appears to have traditional usage for the treatment of insomnia, melancholia, remittent fever, delirium, neurosis, cough etc. The solvent extracts of the drug yielding plants were subjected for preliminary phyto-chemical screening, before evaluation of their biological activity. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc*. The successive extracts of root of *Acorus calamus* revealed the presence of tannins, flavonoids, steroids, glycosides, alkaloids, proteins and phenols. The preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Keywords: *Acorus calamus,* Phytochemical constituents.

1. INTRODUCTION

Phyto-chemicals are the individual chemicals; the plant comprises of, and has the capacity of manufacturing the secondary products. The phyto-chemicals with antioxidant properties, tend to be brightly colored because they contain chromospheres, a series of alternating single-bonded carbons. Isoprene is often the building block of such units, and abundantly found in the chlorophyll and the required antioxidants. Hundreds of phyto-chemicals are currently being studied and are believed to have major positive impact on human health. Important plant secondary metabolites have been isolated over a period of time from natural sources. The phyto-chemicals belong to the following categories such as terpenoids, phenolic compounds, alkaloids, glycosides, carbohydrates, lipids, proteins, nucleic acids, *etc*. (Raaman, 2006).

Binomially named *Acorus calamus* (Sweet flag) (Figure 1) is a seasoning and medicinal herb used in both Indian medicine (Ayurveda) and traditional Chinese medicine for its cognitive properties. *Acorus calamus* is a tall perennial wet land monocot of the Acoraceae family of the genus *Acorus calamus.* The scanted rhizomes (Figure 2) have traditionally been used medicinally and to make fragrances and the dried powdered rhizome (Figure 3) has been used as a substitute for ginger, cinnamon and nutmeg (Raja *et al.,* 2009).

Sweet flag has a very long history of medicinal use in Chinese and Indian herbal traditions. The leaves, stems, and roots are used in various *Siddha* and *Ayurvedic* medicines (Avadhini and Mythili, 2013). It is widely employed in modern herbal medicine, as it has sedative, laxative, diuretic and carminative properties (Balakumbahan *et al.,* 2010; Simonetti, 1990). It is used in *Ayurveda* to counter the side effects of all hallucinogens. Sweet flag, known as "Rat Root" is one of the most widely and frequently used herbal medicines amongst the Chipewyan people (Johnson *et al.,* 1995).

Figure1 *ACORUSCALAMUS*

Figure 2 Rhizomes OF *ACORUS CALAMUS*

Figure 3 POWDER OF *ACORUS CALAMUS*

Both triploid and tetraploid *Acorus calamus* contains alpha-Asarone (Simonetti, 1990) and the other Phyto-chemical includes.

Phyto-chemical screening for the presence of tannin, phlobatannin, saponin, flavonoids, steroids, glycosides, alkaloids, carbohydrates, proteins and phenols, in different solvent systems

was carried out to standardize, the suitable solvent for extraction and the detection of the compounds that is eluted with the solvent. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

2. MATERIALS AND METHODS

2.1. Preparation of extracts

For aqueous extraction, 10g of air-dried powder was added to distilled water and boiled on slow heat for 2h. It was then filtered through 8 layers of muslin cloth and centrifuged for 10 minutes. The supernatant was collected, this procedure was repeated twice. After 6h, the supernatant collected at an interval of every 2h, was pooled together and concentrated to make the final volume one-fourth of the original volume (Parekh *et al.,* 2005). It was then autoclaved at 121^{o}C temperature and at 15 lbs pressure and stored at 4^{o}C. For solvent extraction, 10g of air-dried powder was taken in 100ml of organic solvent (methanol or ethanol) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24h. After 24h the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Parekh *et al.,* 2005) and stored at 4^{o}C in airtight bottles.

2.2. Phyto-chemical screening

Chemical tests were carried out on the aqueous extracts of *Acorus calamus* and on the powdered specimens using standard procedures to identify the constituents eluted in the solvent extracts (Harborne *et al.,*1998).

2.2.1. Test for tannins

About 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green (or) a blue – black coloration.

2.2.2. Test for phlobatannins

Deposition of a red precipitate when an aqueous extracts of each plant sample was boiled with aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

2.2.3. Test for saponins

About 0.5g of the powered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.2.4. Test for flavonoids

Following methods were used to determine the presence of flavonoids in the plant sample. 0.5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of H_2SO_4 (Harborne *et al.,* 1998). A yellow coloration disappeared on standing. Few drops of 1% aluminum solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration observed indicate a positive test for flavonoids.

2.2.4.1. Ferric chloride test

To a small quantity of the alcoholic solution of the extracts a few drops of neutral ferric chloride solution was added. A green colour produced indicates the phenol nucleus.

2.2.4.2. Zinc-hydrochloride acid reduction test

To the alcoholic solution of the extracts a pinch of zinc dust was added and few drops of concentrated hydrochloric acid were added. Magenta color would be produced after a few minutes. Flavanones and dihydro flavanols at the presence of HCl produce bright red color, isoflavonoids and flavones develop yellow, sometimes red colour, and flavonoles do intensive red colour.

2.2.5. Test for steroids

2ml of acetic anhydride was added to 0.5g ethanolic extracts of each sample with 2ml H_2SO_4. The color change from violet to blue or green in same samples indicates the presence of steroids.

2.2.5.1. Salkowski reaction

To the 0.5ml of chloroform extract in a test tube 0.1ml of concentrated sulphuric acid was added from the sides of the test tube. A reddish brown colour observed in chloroform layer indicate the presence of steroids

2.2.5.2. Libermann Buchardt test

To 1ml of petroleum ether extracts in chloroform, 1ml of concentrated sulphuric acid was added followed by the addition of 2ml of acetic an hydride solution and mixed well. A greenish colour developed and turned indicate the presence of steroids.

2.2.6. Test for cardiac glycosides (Keller- Killani test)

5ml of each extracts was treated with 2ml of glacial acetic containing one drop of ferric chloride solution. This was treated with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Harborne, 1973).

2.2.7. Test for alkaloids

2.2.7.1. Mayer's test

The various extracts were distilled in chloroform. The chloroform was acidified and added few drops of Mayer's reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicates the presence of alkaloids.

2.2.7.2. Dragendroff's test

The various extracts were dissolved in chloroform. The chloroform was evaporated and the residue was acidified and added few drops of Dragendroff's reagent (potassium bismuth iodide) appearance for orange red precipitate was observed.

2.2.8. Test for carbohydrates

2.2.8.1. Fehling's test

The extracts with heated with Fehling's A and B solution formation of an orange red precipitate show the presence of reducing sugar.

2.2.8.2. Molisch's test

The extracts with Molisch's reagent and added concentrated sulphuric acid along the sides to form layers. A reddish violet ring at the interference show the presence of carbohydrate. Monosaccharide's give a rapid positive test. Disaccharides and polysaccharides react slower.

2.2.9. Test for proteins

2.2.9.1. Biuret test

The Biuret test is often used to determine the presence of peptide bonds in protein. When a protein reacts with copper (II) sulfate (blue), the positive test is the formation of a violet coloured complex.

2.2.9.2. Ninhydrin test

The Ninhydrin test is a test for amino acids and proteins with a free -NH_2 group. When such an -NH_2 group reacts with ninhydrin, a purple-blue complex is formed.

2.2.10. Test for Phenols

2.2.10.1. Reaction with $FeCl_3$

Phenol gives violet colouration with neutral ferric chloride solution due to the formation of a coloured iron complex, which is a characteristic to the existence of keto-enol tautomerism in phenols (predominantly enolic form).

3. RESULTS

In the present investigation, various solvent extracts of *Acorus calamus* was analyzed for the elution of the significant primary and secondary metabolites to assess the suitability of the solvents and to know the interfering substances present in the particular solvents extract. The results are summarized in (Table 1).

The aqueous and methanolic extracts were tested for flavonoids, tannin, protein, glycosides, phenol, steroids, alkaloids and their presence was confirmed. The tests for carbohydrate, saponin showed the absence of the compounds. (+) is presence of compounds, (-) is absence of compounds in phyto-chemical analysis.

4. DISCUSSION

The investigated plants did not show strong antibacterial activity; however, negative results do not mean absence of bioactive constituents nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed (Taylor *et al.,* 2001). Lack of activity can thus only be proven by using large doses (Farnsworth, 1993). Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents (Jager *et al.,* 1996).

Preliminary phyto-chemical analysis of *Acorus calamus* revealed the presence of alkaloids and flavonoids, though the latter was in lesser amount. The other secondary metabolites like tannins, phenols, steroids, cardiac glycosides, etc were present in trace amounts in some of the extracts, which could contribute to the differences in the activities of extracts, due to phyto-chemical properties and differences among species.

The tests for carbohydrate, and saponin showed the absence of the compounds. Kaushik *et al.,* (2012) reported the presence of carbohydrate, tannin, terpenoids, glycoside, saponin, flavonoid, and the absence of amino acid, alkaloid and steroid. Ashok *et al.,* (2012) reported the presence of

carbohydrate, flavonoid, steroids, glycosides, alkaloids, tannin, and the absence of saponin. Savitha *et al.,* (2010) reported the presence of alkaloid, carbohydrate, protein, glycosides, phenol, tannin, and the absence of amino acid, steroid, saponin and terpenoids, where as in the present studies the extracts showed the presence of alkaloid, flavonoid, tannin, protein, glycosides, phenols, steroids, and the absence of carbohydrate and saponin.

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Table 1. Phyto-chemical analysis of the solvent extracts

S. No	Analysis	Compounds confirmed
1	Tannins	+
2	Saponins	-
3	Flavonoids	+
4	Steroids	+
5	Glycosides	+
6	Alkaloids	+
7	Carbohydrates	-
8	Proteins	+
9	Phenols	+

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SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF $[M(CIN)_2(N_2H_4)_2]$ (M=NI/CD)

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ABSTRACT

Ni (II) and Cd (II) complexes containing hydrazine as ligand and cinnamic acid as co-ligand were synthesized and characterized by hydrazine and metal analyses, thermal analysis and FT-IR spectral studies. It has been found that hydrazine behaves as bidentate ligand and cinnamate as monodentate ligand in the complexes. The biological activities of complexes have been evaluated against two gram negative (Escherichia coli and Pseudomonas aeruginosa) and two gram positive (Bacillus subtilis and Staphylococcus aureus) bacteria by Agar diffusion disc method. It has been found that the complexes have potent activity against the bacteria.

Key words: nanoparticles, XRD, HRTEM, SAED, SEM.

1. INTRODUCTION

The field of bioinorganic chemistry, which deals with the study of role of metal complexes in biological systems, has opened a new horizon for scientific research in coordination compounds. A large number of compounds are important from the biological point of view. Hydrazine was historically used experimentally as a therapeutic agent in the treatment of tuberculosis, sickle cell anaemia, and non-specific chronic illnesses. (Premkumar and Govindarajan, 2005; Yasodhai and Govindarajan, 2000) Hydrazine and their metal complexes have played an important role in the development of co-ordination chemistry. A large number of publications ranging from synthetic to modern physiochemical and biochemical relevant studies of these complexes bear testimony to their importance. Hydrazine carboxylates of the transition metal ions with variety of acids have been reported. These include simple aliphatic mono carboxylic acid, (Sivasankar and Govindarajan, 1995; Sivasankar and Govindarajan, 1997; Sivasankar and Govindarajan, 1994) aliphatic dicarboxylic acids, (Gold, 1987; Govindarajan *et al.,* 1995; Sivasankar, and Govindarajan, 1994; Vikram and Sivasankar, 2007; Vogel, 1985) aromatic mono and dicarboxylic acids (Kuppusamy, and Govindarajan, 1995; Kuppusamy and Govindarajan, 1996) and heterocyclic acids. (Premkumar and Govindarajan, 2006; Von Burg and Stout, 1991) The present paper describes the synthesis, analytical, spectral, thermal studies of Ni (II) and Cd (II) metal complexes containing hydrazine and cinnamic acid.

2. EXPERIMENTAL

2.1. Preparation of $[M(cin)_2(N_2H_4)_2]$

$[M(cin)_2(N_2H_4)_2]$ (M=Ni/Cd) complex was prepared by the addition of an aqueous solution (50 mL) of hydrazine hydrate (1 mL, 0.01 mol) and cinnamic acid (0.74g 0.055 mol) to the corresponding aqueous solution (50 mL) of the corresponding metal nitrates $[Ni(NO_3)_2.6H_2O$, 0.73 g, 0.002 mol or cadmium nitrate hexahydrate, 0.77 g, 0.002 mol] . The complex formed immediately was kept aside for an hour for digestion, then filtered and washed with water, alcohol followed by diethylether and air dried.

2.2. Quantitative methods

The hydrazine content in the complexes was determined by titration using KIO_3 as the titrant (Von Burg and Stout, 1991). The percentage of metals in the complexes was estimated by the standard methods given in the Vogel's textbook (Von Burg and Stout, 1991).

2.2. Physico-chemical techniques

2.2.1. Infrared spectrum

The infrared spectrum of the solid precursor sample was recorded by the KBr disc technique using a Perkin Elmer 597/1650 spectrophotometer.

2.2.2. Thermal analysis

The simultaneous TG-DTA experiment was carried out in Shimadzu DT40, Stanton 781 and STA 1500 thermal analyzer. Thermal analysis was carried out in air at the heating rate of 10°C per minute using 5-10 mg of the sample. Platinum cups were used as sample holders and alumina as reference. The temperature range was ambient to 700°C.

2.2.3. Biological assay

The antibacterial activities of the prepared complexes were determined by the disc diffusion method. The bacteria were cultured in nutrient agar medium and used as inoculum for the study. The antibacterial activity of the synthesized compounds of 25µg, 50µg, 100µg and 200µg concentrations were tested against two gram positive bacteria *Staphylococcus aureus & Bacillus subtilis* and two gram negative bacteria *Pseudomonas aeruginosa & Escherichia coli*. The inhibition zones were calculated and recorded.

3. RESULTS AND DISCUSSION

Table 1 – Compositional analysis data of the prepared complexes

Complex	Hydrazine (%) Found (Calcd.)	Metal (%) Found (Calcd.)	Yield (%)
$[Ni(cin)_2(N_2H_4)_2]$	15.00 (15.36)	14.10 (14.08)	90
$[Cd(cin)_2(N_2H_4)_2]$	13.70 (13.61)	23.60 (23.90)	82

3.1. Chemical formula determination of the complexes

The chemical formula $[M(cin)_2(N_2H_4)_2]$ (M=Ni/Cd) has been assigned to the prepared complexes, based on the observed and calculated percentage of hydrazine and metals, which are found to match closely with the calculated values (Table. 1).

3.2. FT-IR spectral analysis

From the IR spectrum of $[Ni(cin)_2(N_2H_4)_2]$ and $[Cd(cin)_2(N_2H_4)_2]$ complexes, it is observed that the N-N stretching frequency is seen at 962 cm^{-1} and 972 cm^{-1} respectively, which unambiguously proves the bidentate bridging nature of the hydrazine ligand (Sivasankar and Govindarajan, 1996). The asymmetric and symmetric stretching frequencies of the carboxylate ions in $[Ni(cin)_2(N_2H_4)_2]$ are seen at 1612 and 1384 cm^{-1}, respectively with the $\Delta\upsilon$ (υ_{asymm}- υ_{sym}) separation of 288 cm^{-1}. In $[Cd(cin)_2(N_2H_4)_2]$, the asymmetric and symmetric stretching frequencies of the carboxylate ions are seen at 1600 and 1396 cm^{-1}, respectively with the $\Delta\upsilon$ (υ_{asymm}- υ_{sym}) separation of 204 cm^{-1}. From this, the monodentate linkage of carboxylate groups in the complexes is confirmed. The N-H stretching is observed around 3300 cm^{-1} in both the complexes.

Table 2 – FT-IR spectral data of the prepared complexes

Compound	υ N-H cm^{-1}	$\upsilon_{asym(OCO)}$ cm^{-1}	$\upsilon_{sym(OCO)}$ cm^{-1}	$\Delta\upsilon$ cm^{-1}	ν (C=C) cm^{-1}	$\upsilon_{(N-N)}$ cm^{-1}
$[Ni(cin)_2(N_2H_4)_2]$	3351 3270	1612	1384	288	1651	972
$[Cd(cin)_2(N_2H_4)_2]$	3300 3285	1600	1396	204	1638	962

Fig. 1 – FT-IR spectrum of $[Ni(cin)_2(N_2H_4)_2]$

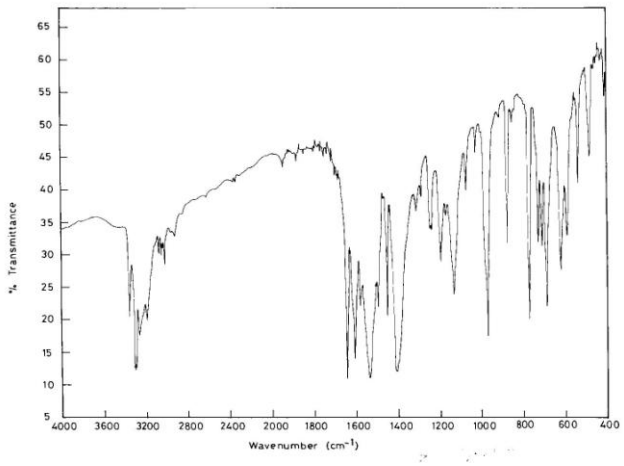
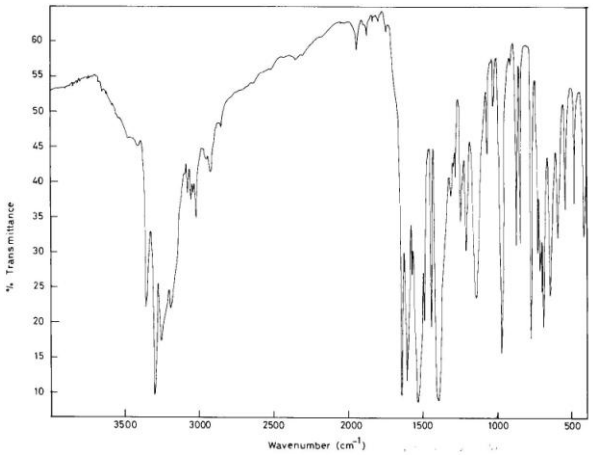


Fig. 2 – FT-IR spectrum of $[Cd(cin)_2(N_2H_4)_2]$



3.3. Thermal analysis

Figure. 3 depicts the TG-DTA curve of the prepared complex $[Ni(cin)_2(N_2H_4)_2]$. The complex undergoes two-step decomposition, the first step being the dehydrazination. In DTA, the corresponding decomposition is observed as an exotherm. The second step is the exothermic decomposition of the dehydrazinated compound, yielding nickel oxide as the final residue with in 250-470°C.

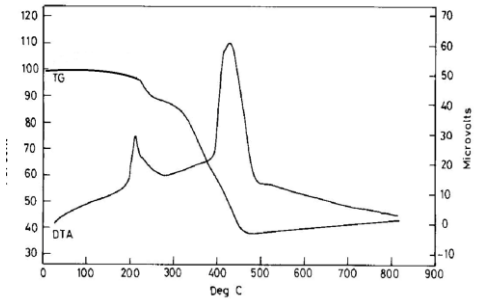


Fig. 3 – TG-DTA curve of $[Ni(cin)_2(N_2H_4)_2]$

From the TG-DTA curve of the cadmium complex shown in figure 4, it is evident that the complex loses weight in three steps. The first step is the endothermic dehydrazination reaction between 166-297°C. In the second step, the dehydrazinated compound gives cadmium acetate as the intermediate exothermically in the temperature range, 297-395°C. Our attempt to separate the intermediate was unsuccessful since the decomposition was continuous. In the third step, the proposed intermediate undergoes exothermic decomposition to give CdO as the end product.

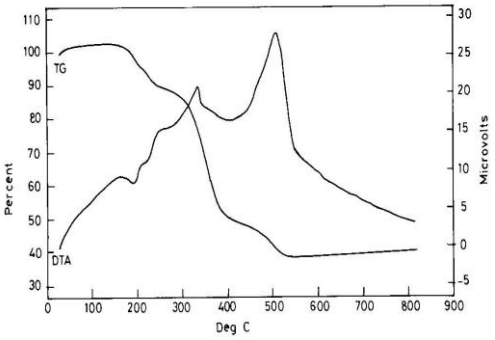


Fig. 4 – TG-DTA curve of $[Cd(cin)_2(N_2H_4)_2]$

Table 3 – Antibacterial activity of the prepared complexes

Complex	Concentration of the complex µg/ml	Zone of inhibition in mm			
		Gram positive bacteria		Gram negative bacteria	
		SA	BS	PA	EC
$[Ni(cin)_2(N_2H_4)_2]$	200	15	14	14	14
	100	12	10	11	11
	50	9	NA	9	8
	25	NA	NA	NA	NA
$[Cd(cin)_2(N_2H_4)_2]$	200	15	14	13	16
	100	13	10	10	13
	50	10	NA	8	10
	25	8	NA	NA	NA

SA - *Staphylococcus aureus;* BS - *Bacillus subtilis;* PA - *Pseudomonas aeruginosa;* EC - *Escherichia coli*

3.4. Antibacterial activity

The antibacterial activity of the synthesised complexes at different concentrations have been studied and recorded in Table 3.

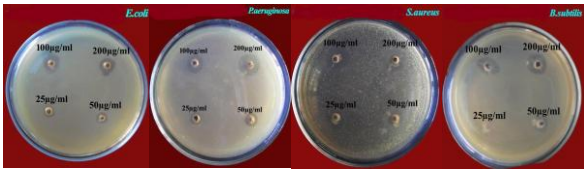


Fig. 5 – Antibacterial activity of $[Ni(cin)_2(N_2H_4)_2]$

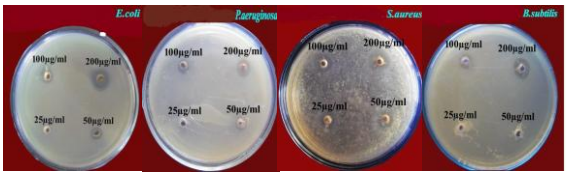


Fig. 6 – Antibacterial activity of $[Cd(cin)_2(N_2H_4)_2]$

It is seen from the figures that the synthesised complexes exhibit antibacterial activity against tested pathogens used in this study. For the Ni (II) complex, in gram negative bacteria the minimum inhibition concentration (MIC) value was exhibited with 50µg/ml for *E.coli and P.aeruginosa*. In gram positive bacteria, the MIC value was identified with 25µg/ml for *S.aureus* and 100µg/ml for *B.subtilis.* For the Cd (II) complex, in gram negative bacteria the minimum inhibition concentration (MIC) value was exhibited with 50µg/ml for *E.coli and P.aeruginosa*. In gram positive bacteria, the MIC value was identified with 25µg/ml for *S.aureus* and 100µg/ml for *B.subtilis.*

4. CONCLUSION

Ni (II) and Cd (II) complexes containing hydrazine and cinnamic acid were synthesized and physico-chemically characterized by FT-IR spectra

and thermal analysis. Antibacterial analysis of the complexes was evaluated among the different bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus.* Among the pathogens, *Staphylococcus aureus* was highly susceptible to both the metal complexes. The present study concluded that synthesised complexes will be used as good drug of choice to manage the bacterial and fungal diseases after evaluating the in-vivo effect of metal complexes on experimental animal and clinical trials.

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Kongunadu Arts and Science College, Coimbatore

IN VITRO EVALUATION OF CRUDE ROOT EXTRACTS OF THE PLANT SPECIES, *HYPOCHAERIS RADICATA* L. FOR POTENTIAL ANTIBACTERIAL ACTIVITY AGAINST SOME HUMAN PATHOGENIC BACTERIA

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ABSTRACT

The antibacterial activity of the crude root extracts of the medicinal plant species, *Hypochaeris radicata* was studied against 15 human pathogen bacteria which includes both Gram-positive and Gram-negative strains by using agar well diffusion method. The chloroform and ethyl acetate extracts displayed broad spectrum activity against all the tested organisms but water extract showed no activity. The petroleum ether and methanol extracts showed only moderate activity. The antibacterial activity of the extracts was compared to the standard Ampicillin. The results of this present study support the plant have good antibacterial potential.

Keywords: *Hypochaeris radicata*, root extract, antibacterial activity, well diffusion method.

1. INTRODUCTION

Finding healing powers in plants is an ancient idea. Over three-quarters of the world population relies mainly on plants and their extracts for health care. The herbal products are today symbolize safety in contrast to the synthetics (Joy *et al.,* 1998). Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Staish, 2008). The effect of plant extracts on bacteria has been studied by many researchers. Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Diallo *et al.,* 1999; Erdogrul, 2002; Rojas *et al.,* 2006).

Hypochaeris radicata belongs to the family, Asteraceae. It is native to Europe and also distributed in high hills of Nilgiris, the Western Ghats, India (above 2000m msl). It has lot of medicinal uses, such as anticancer, anti-inflammatory, anti-diuretic and hepatoprotective activity and to treat kidney problems. The earlier report revealed that this species have a good antioxidant property (Jamuna *et al.,* 2012). The milky sap is bitter and the plant is suspected by some of being unwholesome as fodder (Aarssen, 1981). It is high in protein, calcium, copper, sulphur and chloride. The seed is an important constituent in the diet of many farmland birds including linnets (*Carduelis cannabina*) (Moorcroft *et al.,* 1997). The present study was aimed at to evaluate the antibacterial activity of root extracts of *H. radicata* against 15 pathogenic bacterial strains which include both Gram-positive and Gram-negative types.

2. MATERIALS AND METHODS

2.1. Plant collection and identification

The roots of *H. radicata* were collected from Kattabettu, Nilgiris, the Western Ghats, India (2000m above msl). The authenticity of the plant was confirmed in Botanical Survey of India, Southern Circle, Coimbatore by referring the deposited specimen. The voucher number is BSI/SRC/5/23/2010-11/Tech.153.

2.2. Preparation of plant extracts

The dust free root of *H. radicata* was shade dried and powdered. About 50g of coarsely powdered plant material (50g/250ml) was extracted in a soxhlet apparatus for 8 to 10 hours, sequentially with petroleum ether, chloroform, ethyl acetate, methanol and water separately in order to extract non-polar and polar compounds (Elgorashi and Van Staden, 2004). The extracts obtained were then concentrated and finally dried to a constant weight. Dried extracts were kept at 20˚C until further use.

2.3. Preparation of inoculum

Stock cultures were maintained at 4˚C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient agar medium and were incubated without agitation for 24hrs at 37˚C. The cultures were diluted with fresh nutrient agar broth to achieve optical densities corresponding to $2\text{-}10^6$ colony forming units (CFU/ml) for bacteria.

2.4. Bacterial strains used

In-vitro antibacterial activity was examined for the root extracts of the species, *H. radicata* against 15 bacterial strains which include the Gram-positive strains *viz., Streptococcus faecalis, S. pyogenes, Enterococcus faecalis, Bacillus subtilis, B.*

thuringiensis and *Staphylococcus aureus* and Gram-negative strains *viz., Seratia marcescens, Klebsiella pneumoniae, Proteus vulgaris, P. mirabilis, Salmonella paratyphi, S. parathypi* A, *S. paratyphi* B, *Pseudomonas aeruginosa* and *Escherichia coli.*

2.5. Antibacterial assay

The antibacterial activity of the root extracts was determined by using agar well diffusion method (Cruickshank *et al.,* 1975). The autoclaved media was poured in the sterilized Petri plates. These plates were dried for a period of 20 minutes under aseptic condition before its use. Freshly grown cultures of the tested bacterial strains were streaked over the plates using a platinum wire inoculation loop. On sterile media plates, well of 5.0 mm diameter were punched with the help of a sterile cork borer. The extracts (50µg/mL) were added into the wells by using micropipettes. A standard antibiotic, Ampicillin (50µg/mL) was tested against the pathogens. The plates were incubated at 37ºC for 24hrs. After the incubation period, the diameter of the inhibition zones of each well was measured in millimeter.

2.6. Statistical analysis

The antibacterial activity of *H. radicata* root extracts was indicated by clear zones of growth inhibition. All experiments were performed in triplicate and the results are presented as mean ± SD (Standard Deviation). The significance in the difference of mean was determined according to New Duncan's Multiple Range Test (Gomez and Gomez, 1976).

3. RESULTS AND DISCUSSION

Table 1 shows the effect of various alcoholic and aqueous root extracts of *H. radicata* against the pathogenic bacterial strains studied. Of the five extracts tested chloroform and ethyl acetate extracts showed higher zone of inhibition against all the bacteria. *Salmonella paratypi* B was very sensitive to ethyl acetate (25mm) and chloroform extracts (23mm). This bacterium is a type of germ that is found in the intestines of humans and can cause illness. Symptoms happen gradually which may include: fever (usually as high as 103 or 104˚F), headache, fatigue (feeling tired), diarrhea (sometimes bloody), stomach pain, serious complications can occur (septicaemia, meningitis), especially in young children and immunocompromised patients (Nagano *et al.,* 2006). Next to that, the another bacterium, *Proteus mirabilis* was very susceptible to ethyl acetate (23mm) and chloroform (23mm) extracts. This bacterium is one of the common species of Enterobacteriaceae and well known as a pathogen in urinary tract infections (Warren *et al.,* 1982; Warren *et al.,* 1987). It has been implicated in hospital outbreaks and cases of cross-infection. The zone of inhibition ranged between ≥ 9-15mm is an indication of strong antimicrobial activity (Rani *et al.,* 2004). The petroleum ether and methanol extracts showed only lesser activity. However, in water extract no activity was found. Several workers have reported that water extract do not have much activity against bacteria (Martin, 1995; Paz *et al.,* 1995; Vlietinck *et al.,* 1995). Significant antibacterial activity was found in chloroform and ethyl acetate extracts as compared with the standard drug, ampicillin against *Proteus mirabilis* and *Salmonella paratyphi* B.

From the above results, it can be concluded that the antibacterial activity of the chloroform and ethyl acetate extracts of *H. radicata* exhibited greater activity than that of the other solvent extracts attempted. It may be due to the greater solubility of the phytochemical compounds in these organic solvents (De Boer *et al.,* 2005). Except the water extract all the other extracts showed better antibacterial activity against all the bacterial strains with average zone of inhibition ranged from 6mm to 25mm. The present results revealed that the extract of *H. radicata* was effective against both Gram-positive and Gram-negative bacteria. Inhibition mechanisms of plant extracts against bacteria were by interfering with protein synthesis in bacteria, degrading the existing present cell wall or interfering with bacterial cell wall synthesis, and damaging cell membrane of bacteria also (Yasni, 2009). Previous studies reported that many Asteraceae members exhibited greater biological activity (Ahlem *et al.,* 2008, Malarkodi and Manoharan, 2013). According to this study, plant based antibacterial drugs have enormous therapeutic potential as they can serve the purpose with less side effects that are often associated with synthetic antimicrobial agents. The present study provide the scientific information on the plant extract of *H. radicata* and support the usage of root of this plant for curing many bacterial diseases.

5. CONCLUSION

The results presented in this study conclusively demonstrate the antibacterial potential of root extracts of *H. radicata*. The chloroform and ethyl acetate extracts justified the uses of curing the infectious diseases caused by pathogenic microbial strains. Hence, it could be used as a source for pharmaceutical substances with antibacterial potential. Further work is aimed at to isolate and identify the specific compounds for the antibacterial agents.

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Table 1. Antibacterial activity of the root extracts of *Hypochaeris radicata* against pathogenic bacteria.

S.No	Name of the organisms	Diameter of the inhibition zone (mm)					
		Control*	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
	Gram-positive Bacteria						
1.	*Streptococcus faecalis*	22.00 ± 0.10^{a}	06.00 ± 0.10^{b}	14.00 ± 0.57^{c}	15.68 ± 0.13^{c}	08.00 ± 0.10^{b}	-
2.	*S. pyogenes*	13.00 ± 0.50^{a}	05.33 ± 0.58^{b}	9.33 ± 0.58^{bc}	12.67 ± 0.52^{c}	10.67 ± 0.53^{bc}	-
3.	*Enterococcus faecalis*	20.33 ± 0.58^{a}	07.33 ± 0.28^{b}	12.67 ± 0.53^{bc}	14.00 ± 0.20^{c}	10.00 ± 0.15^{bc}	-
4.	*Bacillus subtilis*	18.67 ± 0.21^{a}	06.00 ± 0.10^{b}	15.67 ± 0.51^{c}	10.67 ± 052^{bc}	8.67 ± 0.15^{bc}	-
5.	*Bacillus thuringiensis*	23.00 ± 0.20^{a}	10.00 ± 0.20^{b}	13.00 ± 0.10^{c}	11.67 ± 0.53^{bc}	10.00 ± 0.10 ^{b}	-
6.	*Staphylococcus aureus*	22.67 ± 0.40^{a}	08.67 ± 0.52^{b}	18.00 ± 0.65^{c}	15.67 ± 0.06^{c}	09.67 ± 0.53^{b}	-
	Gram-negative Bacteria						
7.	*Seratia marcescens*	18.38 ± 0.16^{a}	07.67 ± 0.58^{b}	13.33 ± 0.58^{ab}	9.67 ± 0.89^{b}	09.00 ± 0.10^{b}	-
8.	*Klebsiella pneumoniae*	21.00 ± 0.20^{a}	06.00 ± 0.10^{b}	16.67 ± 0.51^{c}	16.33 ± 0.16^{c}	09.67 ± 0.58^{b}	-
9.	*Proteus vulgaris*	21.00 ±0.10^{a}	06.67 ± 0.53^{b}	12.00 ± 0.20^{c}	10.33 ± 0.58^{bc}	09.00 ± 0.10^{bc}	-
10.	*P. mirabilis*	21.33± 0.15^{a}	08.33 ± 0.60^{b}	23.33 ± 0.89^{a}	23.00 ± 0.20^{a}	09.67 ± 0.53^{b}	-
11.	*Salmonella paratyphi*	21.67 ± 0.58^{a}	06.00 ± 0.10^{b}	11.00 ± 0.10^{c}	9.67 ± 0.58^{c}	07.33 ± 0.58^{b}	-
12.	*S. paratyphi* – A	20.33 ± 0.58^{a}	05.33 ± 0.58^{b}	17.00 ± 0.20^{c}	18.67 ± 0.21^{c}	09.00 ± 0.10^{b}	-
13.	*S. paratyphi* – B	22.33 ± 0.51^{a}	09.33 ± 0.53^{b}	23.00 ± 0.20^{a}	25.00 ± 0.20^{a}	09.00 ± 0.10^{b}	-
14.	*Pseudomonas aeruginosa*	19.67 ± 0.89^{a}	08.67 ± 0.89^{b}	12.00 ± 0.30^{c}	10.67 ± 0.53^{bc}	09.67 ± 0.53^{bc}	-
15.	*Escherichia coli*	20.00± 0.46^{a}	06.00 ± 0.73^{b}	10.33 ± 0.58^{c}	10.67 ± 0.80^{c}	11.00 ± 0.65^{c}	-

*Ampicillin, '-' indicates no activity.
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

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EVALUATION OF ANTIBACTERIAL POTENTIAL OF THE TRADITIONAL MEDICINAL CLIMBER, *SOLENA AMPLEXICAULIS* (LAM.) GANDHI. (CUCURBITACEAE)

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ABSTRACT

Solena amplexicaulis (Cucurbitaceae) is a traditional medicinal climber generally prescribed for wound healing by the local healers in western districts of Tamil Nadu. The aim of the present study was focused on investigating the potential of antibacterial activity via *in vitro* approach. The aqueous and organic solvent extracts (hexane, benzene, chloroform and methanol) of the stem part of *S. amplexicaulis* were tested against 15 human pathogenic bacteria by agar well diffusion method. Results showed promising antibacterial activity against the bacteria tested. Among them, chloroform and benzene extracts were found to have more potent inhibitory effect in comparison to the other extracts. It proves the therapeutic importance of the species in curing infectious diseases and encouraged for its extensive use in health care practices.

Key words: *Solena amplexicaulis,* antibacterial activity, agar well diffusion method.

1. INTRODUCTION

Phytomedicines play a major role in human health care system and a source of great economic value all over the world (Ahmad *et al.,* 1998). These medicinal plants represent a rich source of antimicrobial agents, used medicinally in different countries and as a source of many potent and powerful drugs (Uniyal *et al.,* 2006). These beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant (i.e.) alkaloids, steroids, tannins, phenolics, flavonoids etc which are capable of producing definite physiological action on body (Bishnu *et al.,* 2009). As per the World Health Organization (WHO) reports, 80% of the world populations are presently using herbal medicine for some aspects of primary health care. Many pharmaceutical companies are also showing interest in manufacturing plant derived drug based on their belief of 'Green Medicine' is safe and more dependable than the costly synthetic drugs, which have adverse side effects (Sujatha, 2005).

Solena amplexicaulis (Cucurbitaceae) is a perennial dioeceous climber with tuberous root found throughout Asia mainly in hilly dry deciduous forests and scrub jungles. It has been used in traditional Indian medicine for various ailments like spermatorrhoea, thermogenic, appetizer, cardiotonic, diuretic and haemorrhoids (Kritchevsky, 1978) and its leaves have good anti-inflammatory activity and also recommended for skin lesions and other skin diseases (Arun *et al.*, 2011). The leaf juice is taken orally to cure jaundice (Mohammed *et al.*, 2011). The fresh stem is externally used to promote the conception (Ignacimuthu *et al.,* 2008). Unripe fruits are eaten raw to strengthen the body (Jeyaprakash *et al.,* 2011) and used as vegetable also. It helps to increase the secretion of milk during lactation and also used as expectorant, antidepressant and antianxiety and to reduce asthmatic conditions. (Bandyopadhyay and Sobhan Kr Mukherjee, 2009). Root is a stimulant and purgative (http://hpforest.nic.in/potter.htm). The decoction of the root is taken orally to cure stomachache (Abdolbaset *et al.,* 2011). The whole plant is determined to be a potential source of natural antioxidant activity (Venkateshwarlu *et al*., 2011 and Karthika *et al.,* 2012) and also used for the treatment of diabetes (Pullaiah *et al*., 2003).

Considering the indigenous uses of this species, the present investigation was taken up with an objective to evaluate the antibacterial potential of stem extract against certain human pathogenic bacteria that may provide scientific justification to the traditional uses in treating various ailments.

2. MATERIALS AND METHODS

2.1. Plant material

The stem of *S. amplexicaulis* was collected from Madukkarai, Coimbatore district, Tamil Nadu. Collected plant materials were washed thoroughly in tap water, shade dried and then homogenized to fine powder and stored in air tight bottles.

2.2. Preparation of extracts

About 50g coarsely powdered plant material (50g/250ml) was extracted in the soxhelt extractor for 8 to 10 hours, sequentially with hexane, benzene, chloroform, methanol and water. Then each extract was evaporated to dryness.

2.3. Bacterial strains

In vitro antibacterial activity was examined for the crude extracts of stem of the study plant, against 15 bacterial species which include the Gram positive strains *viz., Streptococcus faecalis, S. pyogenes, Bacillus subtilis, B. thuringiensis, Staphylococcus aureus* and *Enterococcus faecalis* and Gram negative strains *viz., Klebsiella pneumoniae, Salmonella paratyphi, S. paratyphi* A, *S. paratyphi* B, *Escherichia coli, Proteus vulgaris, P. mirabilis, Serratia marcescens* and *Pseudomonas aeruginosa.*

All these bacterial strains were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. All the bacteria were maintained at 4°C on nutrient agar slants for further use.

2.4.Bacterial susceptibility testing

An inoculum of each of the pathogenic bacterial strains was suspended in 5ml of nutrient broth and incubated at 37°C for 18 hrs (10^6-10^8 bacteria $CFUml^{-1}$). Bioassay was carried out by using agar well diffusion method (Perez *et al*., 1990; Murray *et al*., 1995; Olurinola, 1996). Inoculum was spread over Muller – Hinton agar medium with sterile glass spreader. A well of 6 mm diameter was made using a sterile cork borer and filled with 50µl of different extracts by using micropipette in each well in aseptic condition. The plates were kept at room temperature for absorption of extract in the medium and further incubated in an incubator at 37°C for 24 hrs. The antibacterial activity was evaluated by measuring the diameter of inhibition zone (mm). Ampicillin was used as positive control (50µl/ml) and DMSO (Dimethyl sulphoxide) as negative control (50µl).

2.5. Statistical analysis

All the analyses were done in triplicate and results were expressed as mean±SD. The data were subjected to one way analysis of variance (ANOVA) and the significance of the difference between mean was determined by Duncan's Multiple Range Test with significance level, $P<0.05$. ANOVA was performed using the statistical software SPSS (SPSS Inc. Chicago, USA).

3. RESULTS AND DISCUSSION

Agar well diffusion method is widely used in *in vitro* method of evaluation of antimicrobial activity of various chemicals. The size of the inhibition zone depends on the solubility of the test material, time and temperature of incubation (Weiss *et al.,* 1996). In the present study the antibacterial activity of aqueous and solvent extracts (hexane, benzene, chloroform and methanol) of stem part of *S. amplexicaulis* against certain human pathogenic bacterial which include both gram positive and gram negative in terms of inhibition ability were presented in Table – 1. Among the five extracts, benzene and chloroform extracts showed broad spectrum of antibacterial activity in comparison to hexane and methanol extracts which showed mild activity with the zone of inhibition diameter ranged from 8 - 24mm and 6 – 12mm, respectively. The water extract showed almost no activity. Different solvents have been reported to have different capacity to extract phytoconstituents according to their solubility or polarity, and most of the compounds are dissolved well in alcoholic solvents than in water (Marjorie, 1999). The antibacterial activity was compared with the inhibitory activity of antibiotic, Ampicillin which showed varied inhibitory zones of 13 – 24 mm. The negative control DMSO, which showed no zone of inhibition. Among the 15 bacteria, *Staphylococcus aureus* of Gram positive type showed higher susceptibility to stem extract than the antibiotic, ampicillin. *S. aureus* is a well known wound pathogen (Lullmann *et al.,* 2000) which can be found as the part of the normal skin flora and in the nasal passages (Kluytmans *et al.,* 1997 and Cole *et al.,* 2001). In the study, Gram positive bacteria are little bit sensitive than that of Gram negative bacteria because the latter are frequently reported to have developed multidurg resistance due to their outer membrane which act as a barrier to many environmental substances, including antibiotics (Tortora *et al.,* 2001; Johansson *et al.,* 2011; Johnson *et al.,* 2011; Ramakant *et al.,* 2011). The zone of inhibition ≥ 9-15mm is an indication of strong antimicrobial activity (Rani and Khullar, 2004). So, the stem extract of *S. amplexicaulis* having the potential for killing the bacteria. High antibacterial effects of alcoholic extracts of certain Cucurbitaceae members were already reported well *Trichosanthes cucumerine* (Arawwawala *et al.,* 2011), *Citrullus colocynthis* (Gurudeeban *et al.,* 2010) and *Coccinia grandis* (Farrukh *et al.,* 2008)).

5. CONCLUSION

The present research is a right step to the direction of searching novel and more effective antibacterial compounds in plants. In conclusion the species, *S. amplexicaulis* extracts exhibited antibacterial activity against both Gram positive and Gram negative bacterial strains mediating the presence of a broad spectrum of antibacterial compounds. This study suggests that further research will be needed for pharmacological aspects and elucidate the specific phytoactive compounds in the stem extract of *S. amplexicaulis* and hence to go for commercial application through pharmaceutical industries.

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Table 1: *In vitro* antibacterial activity of stem extract of *Solena amplexicaulis* by agar well diff method.

S.No	Name of the bacteria	Diameter of inhibition zone (mm)					
		Control*	Hexane	Benzene	Chloroform	Methanol	Water
	Gram positive						
1.	*Streptococcus faecalis*	24.33±0.15^{a}	-	11.33±0.93^{b}	12.33±0.52^{b}	9.00±0.10^{c}	-
2.	*S. pyogenes*	20.00±0.81^{a}	6.67±0.53^{b}	11.33±0.16^{c}	13.67±0.23^{ac}	8.33±0.51^{bc}	-
3.	*Bacillus subtilis*	23.33±0.21^{a}	6.67±0.58^{b}	14.33±0.63^{c}	16.33±0.72^{c}	8.33±0.89^{b}	6.82±0.29^{b}
4.	*B. thuringiensis*	18.33±0.58^{a}	8.00±0.65^{b}	8.00±0.20^{b}	12.67±0.44^{c}	7.67±0.53^{b}	-
5.	*Staphylococcus aureus*	19.67±0.53^{a}	11.33±0.21^{b}	24.33±0.59^{c}	23.67±0.15^{c}	11.33±0.08^{b}	-
6.	*Enterococcus faecalis*	20.00±0.65^{a}	6.33±0.31^{b}	13.33±0.16^{c}	18.00±0.36^{ac}	10.00±0.30^{bc}	-
	Gram negative						
5.	*Klebsiella pneumonia*	15.00±0.10^{a}	-	13.00±0.81^{a}	15.61±0.81^{a}	7.67±0.79^{b}	-
6.	*Salmonella paratyphi*	23.00±0.73^{a}	-	9.67±0.53^{b}	11.33±0.57^{c}	8.00±0.26^{b}	7.67±0.62^{b}
7.	*Salmonella paratyphi* A	16.00±0.20^{a}	6.67±0.28^{b}	11.00±0.10^{c}	13.67±0.58^{c}	7.33±0.11^{b}	-
8.	*S. paratyphi* B	13.33±0.58^{a}	8.33±0.15^{b}	13.33±0.15^{a}	17.00±0.20^{c}	10.00±0.20^{ab}	-
9.	*Escherichia coli*	16.33±0.23^{a}	8.00±0.65^{b}	13.67±0.36^{c}	16.00±0.61^{a}	9.00±0.10^{b}	-
10.	*Proteus vulgaris*	19.00±0.73^{a}	8.33±0.28^{b}	16.67±0.51^{c}	17.67±0.64^{ac}	12.33±0.41^{bc}	-
11.	*P. mirabilis*	19.00±0.36^{a}	6.50±0.35^{b}	12.33±0.44^{c}	13.67±0.53^{c}	7.67±0.53^{b}	-
12.	*Serratia marcescens*	18.00±0.20^{a}	7.67±0.31^{b}	18.00±0.20^{a}	17.00±0.30^{a}	9.33±0.58^{c}	-
13.	*Pseudomonas aeruginosa*	16.67±0.89^{a}	7.67±0.52^{b}	10.00±0.65^{c}	14.67±0.31^{ac}	9.67±0.15^{bc}	-

'*' Amphicillin, '-' indicates no activity.
Values were performed in triplicates and represented as mean±SD.
Mean values followed by different superscript in a column are significantly different (P<0.05).

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TAXONOMIC NOTES ON *BOUCEROSIA TRUNCATO-CORONATA* SEDGEWICK – A LITTLE KNOWN PLANT SPECIES IN SOUTHERN INDIA

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ABSTRACT

Boucerosia truncata-coronata Sedgewick is a little known endemic succulent species in southern India. The description of a new species *Boucerosia nilagiriana* Subba Rao and Kumari from Nilgiri hills make an attention to study the *B. truncata-coronata*. The recent study proved that *B. nilagiriana* is conspecific to *B. truncato-coronata*. This communication provided detailed description, taxonomic notes and illustration for easy identification and conservation of the little known plant species.

Keywords: Taxonomic notes, *Boucerosia truncate-coronata,* endemic plant.

1. INTRODUCTION

The genus *Boucerosia* Wight belongs to the subtribe Ceropegiae of the family Apocynaceae and comprises about 100 species distributed in Africa, Arabia, Afghanistan, Pakistan, India, Sri Lanka and Myanmar (Plowes, 1995). In India this genus is represented by about 14 species distributed in two distinct centres of distribution: the northern Indo-Pakistan border areas and southern Peninsular India.

Peninsular India harbours 8 species of *Boucerosia*, of which *B. lasiantha, B. campanulata* are synonymised under *B. umbellata*, whereas *B. nilagiriana* and *B. crenulata* are synonymised under *B. truncato-coronata* (Meve and Leide, 2002). Of these 8 species, only 5 species are now treated at species level and among them 4 species are confined to the southern India. *B. umbellata* has its distribution extended to Sri Lanka. They occur in narrow isolated habitats especially in arid areas of scrub forests, rocky foot hills and dry lands among the cultivated fields of both in Eastern and Western Ghats slopes. Some of these species are narrow endemic and extremely rare while a few are so far known only from their original descriptions. *Boucerosia truncato-coronata* is one such little known endemic species which was first described by Sedgewick in 1921 near Hubli in Dharwar district of Karnataka. The type specimen was not preserved in any herbaria and also the protologue has not mentioned any types. Gilbert (1990) designated specimen Sedgwick. *s.n*. Sept. 1919 from India, Bombay, Dharwar district, Hubli as Lectotype. Gravely and Mayuranathan (1931) have included this taxon in their monograph of Indian *Caralluma* by quoting Sedgwicks' collection made from areas of Bombay presidency. Later, Kumari and Subba Rao (1974) have described a new species of *Caralluma* (*Boucerosia*) *nilagiriana* from Nilgiri hills, which is closely related to *B. tuncato-coronata*. They have collected unflowered specimen from Nilgiri hills and described it after flowering by planting in the garden. It is now treated as synonym for *B. truncato-coronata*. Unfortunately both the specimens are not available in any of the Indian herbaria for further critical studies. After Sedgewick's original report, *B. trucato-coronata* was neither recorded from its original habitat in Dharwar district, nor elsewhere. This perhaps prompted Ahmedullah and Nayar (1987) and Nayar (1996) to categorize this species in danger of extinction in its habitat. The conservation status of this taxon has also not been evaluated so far.

During a recent field survey carried out in connection with a research project on 'Taxonomic survey of Indian genus *Caralluma'*, *B. truncato-coronata* was collected in Tumkur area of Karnataka, 87 years since its first discovery by Sedgewick in 1921. A small population comprising about 50 individuals was located on rocky boulders among the cultivated lands near Pemmonalli village on the way of Devarayan Durga.

The only source of information available on the morphology and geographical distribution of the species are the original latin description provided by Gravely and Mayuranathan (1931) followed by a brief description in English by Jagtap and Singh (1999). Therefore a more detailed description along with relevant illustration are provided to help easy identification of this little known species.

Boucerosia truncato-coronata Sedgwick, J. Ind. Bot. 2: 125. (1921). Type: Lecto type was designated by Gilbert 1990. *B. nilagiriana* Kumari & Subba Rao,

J. Bombay Nat. Hist. Soc. 73: 199 (1976); Plowes, Haseltonia 3: 59. (1995). *B. crenulata* (Wall.) Wight & Arn. Contr. Bot. Ind. 34. (1834). *Caralluma truncato-coronata* (Sedgwick.) Gravely & Mayurn. Bull. Madr.Gov. Mus. N. S. 4(1): 21 (1931). **(Fig. 1)**.

Plants growing in clumps, up to 15 cm high, with subterranean runners, spreading by suckers. Branches slender, prostrate, rooting at nodes, not tapering, 4-angled, c.6 mm wide, laterally furrowed. Leaves minute, ovate, glabrous, tip acute, arise on the tubercles of the angles. Inflorescence terminal, umbellate, 10-13-flowered. Flowers campanulate, pentagonal, apically flattened in bud. Bracts 2, minute, c. 2.5 mm long, linear. Bracteoles inconspicuous. Pedicels c. 17 mm long, green. Calyx divided up to the base, segments 5, alternating with corolla lobes, c. 4 mm long, linear, acute, glabrous. Corolla c. 8.5 mm across, corolla-tube c. 6 x 6 mm, campanulate, segments 5, outside green mottled with purple, three distinct colouration on the tube inside, a deep purple ring at the middle with dark purple ring formed a rim of mouth and interior tubes light purple dotted white background with valvate limb, 5-fid, with pellucid glands at bottom. Corolla-lobes distinct, triangular, acute at tip, dark purple to brownish purple above, a few thin yellow transverse lines interrupted on the lobes, 3 or 4 golden yellow hairs on the margins at the base of corolla lobes. Corona c. 4 mm in diameter, dark purple; outer corona 5-lobed, truncate tips, lobes prominently cuspidate on either side; inner lobes ligulate, elongate, ascending and appressed on dorsal sulcate surface of anthers; staminal column short, incumbent on the stigma; anthers light yellow, lobes broadly elliptic. Pollinia solitary, erect, yellowish red, compressed, oblong or obtuse, faintly transversely striped, attached laterally to the erect purple corpusculum long, glabrous; stigma 5-angled. Follicle paired, erect, c.5 cm long, lanceolate, glabrous, light purplish streaks above, slightly beaked at the tips.

Flowering & Fruiting: November – March.

Specimen examined: Karnataka, Tumkur district, Pemmonalli hills, near cultivated lands on the way of Devarayan Durga, S. Karuppusamy and A. Ugraiah 31768 (SKU); Tamilnadu, Coimbatore district, Madhukarai, Kurudimalai, S. Karuppusamy and A. Ugraiah 31733 (SKU).

There are no herbarium specimens in any Indian herbaria except *B. nilagiriana* Kuamri & Rao 39262A (CAL), 39262, 39263, 38287 and 37329 (MH). The present collection made after a long gap of type collection approximately 400 km away from type locality. A fragmented population was observed in about 50 km surrounding areas of Tumkur district of Karnataka.

Distribution: Karnataka and Tamilnadu.

Uses: Locally this species is used as vegetable as raw material.

2. TAXONOMIC NOTES ON BOUCEROSIA

Genus *Boucerosia* was first discovered in 1834 by Wight and Arnott with flowers in terminal umbels. It was used quite widely for Indian, Arabian and Mediterranian Stapeliad species until N.E. Brown (1892), who included *Boucerosia* under the genus *Caralluma,* perhaps overwhelmed by the variety of species being discovered latter in Southern India combined with coronal variations, stem characters, striations and hairiness patterns on flowers. Brown made no attempt to recognize infrageneric groupings of the taxa. Schumann (1895) recognized three sections under *Boucerosia* which are *Eucaralluma* (=*Caralluma*), *Lalacruma* (=*Caralluma gracilis*) and *Boucerosia*, but completely ignored that subgenus *Boucerosia* is restricted only to Southern India, Sri Lanka and Myanmar.

In southern India, there is only one widespread Stapeliad species viz. *Boucerosia umbellata*. This species occurs over much of the subcontinent south of Madhya Pradesh including Orissa, Maharashtra, Karnataka, Andhra Pradesh, Kerala, Tamilnadu and also extends to Sri Lanka (Gilbert, 1990). After Brown (1810), the genus has not got any attention of taxonomists, but the first review on *Caralluma* and its segregates came in 1990 by Gilbert, he has segregated the succulent stemmed stapeliads into three subgenera viz. *Caralluma, Urmalcala* and *Boucerosia*. He has enumerated many synonyms for the *Boucerosia* such as *Hutchinia* (Wight & Arnott, 1834), *Apteranthes* (Miken, 1835), *Sarcodon* (Brown, 1878), *Desmidorchis* (Munster, 1990) and *Aperanthes* (Munster, 1990). Gilbert has considered *B. truncato-coronata* as a individual species but Plowes (1995) suggested that it is conspecific to *B. crenulata*. But he noted *B. nilagiriana* as a synonym for former species. He has given the note for above comment based on J.S. Sarkaria's personal consideration but it has not appeared in any paper.

Recently molecular taxonomic study was conducted on subtribe Ceropegiae by Meve and Liede (2002). They have erected a separate clade for genus *Boucerosia*, which included only seven taxa and also included another one closely related Indian genus *Frerea* under *Boucerosia*. All the above studies of Gilbert (1990), Plowes (1995) and Meve and Liede (2002) have clearly mentioned that *Boucerosia*

complex of India must be revised for their taxonomic treatments. The present investigation has concentrated on taxonomic treatment of *B. truncato-coronata* and also the study reported rediscovery of the same taxon from Karantaka nearly 400 km far from type locality. Even though Sedgwick has not mentioned precise locality for *B. truncato-coronata*. According to his description, all the characters coincide with our recently collected specimens.

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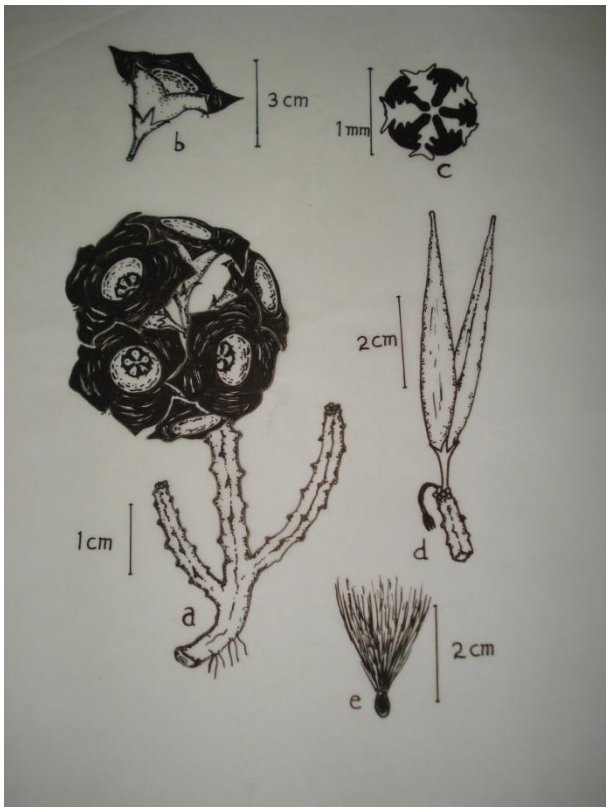
Figure 1. *Boucerosia truncato-coronat* Sedgewick



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A SURVEY OF USEFUL AQUATIC MACROPHYTES, AND ITS BIOLOGICAL SPECTRUM IN KURANDIKULAM, MELASANKARANKUZHI, KANYAKUMARI DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

This paper pen down the survey of useful aquatic macrophytes, and its biological spectrum inKurandikulam, Melasankarankuzhi, Kanyakumari District, Tamil Nadu, India.

Keywords: Aquatic macrophytes, Biological spectrum, Kanyakumari.

1. INTRODUCTION

Plants are vital to the function of aquatic ecosystems for their role in providing food, oxygen and habitat for other organisms. Aquatic macrophytes respond to the changes in water quality and have been used as bioindicator of pollution (Tripathi and Shukla, 1991) and are frequently used to reduce different kinds of pollutants from polluted water. The growth of aquatic plants or macrophytes in aquatic ecosysystem has an important influence on both the structure and the processes that occur in the stream habitat. Studies on aquatic macrophytes are in its initial phase in South India and it requires immediate attention (Gopal and Zutchi, 1998; Udayakumar and Doss, 2010). Rivers, soil moisture and relatively shallow groundwater basins are the principal sources of water for human (Srivastava*et al.,* 2008). One percent of the world's surface is covered by various fresh water habitats including the ponds (Gleick, 1996). They support life of 7 % of the estimated 1.8 million described species. Aquatic macrophytes can be used as a tool in the determination of pollution and nutrient level (Melzer, 1999) water quality and lake condition (Clayton and Edwards, 2006) trophic status of lakes (Palmer *et al.,* 1992), pollutant degradation (Mccutchen and Scgnoor, 2003) and decontaminate waste water (Nahlik and Mitsch, 2006). Urbanisation, industrialisation and bursting human population are the major threats to the fresh water ecosystem. In the present study, an attempt has been made to investigate the qualitative aspects of the macrophytes of Kurandikulam, Thoppur, kanyakumari District. Moreover, the present study is the first attempt to document the macrophytic diversity of the Thoppur, Kanyakumari District.

2. METHODOLOGY

2.1. Study Area:

The study area Kurandikulam is located in Melasankarankuzhipanchayat. This pond is situated in northern part of the Melasankarankuzhi. The villages like, Kurandi, Vadalivilai, Kothavilai are surrounding this pond. Most of the people are coolies. Around 2000 people are depends this pond for their domestic purposes. Due to the anthropogenic activities, this pond area is shrined day by day.

An extensive floristic survey was conducted during the year June 2012 – November 2012. The plant specimens were collected to prepare herbarium specimens and authenticate their correct identities. The collected specimens were identified taxonomically with the help of available monographs, taxonomic revisions and floras (Gamble and Fischer, 1915-1935; Mohanan and Henry, 1994; Santhapu and Henry, 1994) and collected specimens were cross checked for correct identification at the Herbarium of Tropical Botanical Garden and Research Institute, Trivandrum, Kerala and Department of Botany, S.T. Hindu College, Nagercoil.

3. RESULTS AND DISCUSSION

In the present study, altogether 65 macrophytic species were recorded and species were grouped under different categories i.e, Marginal (30 species), sub-merged (9 species), floating (7 species) and emergent (19 species) recorded in the present study were distributed over genera and families. Dicots (37 species) were dominant (19 families) and covered 57% of the total number of macrophytic species. Monocots (28 species) (9 families) were next to dicots and covered 43% of the total plants. Algae, Bryophytes and Pteridophytes were poor in distribution and were represented by three species each. Scientific names and habitat status are given in Table-1. Table-2 depicts the family wise distribution of genus and species and their percent composition and Table-3

depicts the summary of Taxonomic data and Table – 4 showed the habitat status (number wise) their uses in Table-5. Life form classification and the Biological spectrum of the identified plants were showed in Table 6, 7.

From the present study, it was evident that study area was clearly dominated by dicotyledons followed by the Monocots, but Algae, Bryophytes and Pteridophytes contributed only three species each.Out of 65 species, the family Cyperaceae had contributed 7 species and their percentage composition is 10.6%. Asteraceae, Euphorbiaceae, Lamiaceae, Hydrocharitaceae and Charophyceae had 3 species and their percentage composition is 4.5%. Convolvulaceae, Caesalpiniaceae, Poaceae, Salviniaceae, Polytrichaceae had two species and their percentage composition is 3%. Boraginaceae, Capparaceae, Nelumbonaceae, Nymphaceae, Onagraceae, Papaveraceae, Sapindaceae, Scrophulariaceae, Araceae, Ceratophyllaceae, Lemnaceae, Postederiaceae, Potamogetonaceae, had one species each and their percentage composition 1.5%. The diverse type of macrophytic forms recorded from the study area indicates that the selected area is very rich in plant diversity particularly in submerged and emergent species. *EichhorniaCrassipes*(free floating),*Hydrillaverticillata* (submerged) and *Cynodondactylon* (marginal) *Cyperusrotundus* (emergent), *Commelinabenghalensis* (emergent), *Acalyphaindica*(marginal) and *Tridaxprocumbens* (marginal) were the dominant species in the study area. The growth of both submerged and emergent species was governed to some extent by the magnitude and duration of water depth. Most of submerged species like *Utricullariastellaris, Ceratophyllumdemersum, Potamogetonmalanius, Hydrillaverticillata*and *Nitella* species grew luxuriantly in shallow water as light and nutrients are available in plenty.Rooted floating leaved plant like*Lemna minor, Azollapinnata, Salvinianatans* and *Marchantia*also favoured this study area. Emergent like *Alternantherasessilis, Scopariadulcis, Marsileaquadrifolia*and *Ipomeaaquatica* were recorded from continuously water logged area of the study area. Some of the emergent like *Ecliptaalba, Euphorbia hirta* and *Cyperusiria* were found on dry area near the study area.

In the present study, the Raunkiar's system of life form classification has been followed.The identified plants were categories as Phanerophytes, Chamaephytes, Hemicryptophytes, Geophytes and Cryptophytes. Presently observed species include 7.69% Chamaephytes which showed -38.31 deviation from normal biological spectrum; 15.38% phanerophytes and showed deviation of +6.38; 6.15% Cryptophytes and exhibits a deviation of +0.15%; 3.07% Hemi cryptophytes with a deviation of -22.93%; 67.69% Therophytes and the deviation from normal biological spectrum is +54.69(Table -7). There are five plant species belongs to Chamaephytes (*Polygonumglabrum*, *P. barbarum*, *Charazeylanica*, *C. Nitzii and Nitella hyaline*). Forty four plant species belongs to Therophytes, ten species belongs to Phanerophytes, four belongs to Cryptophytes and two plants belongs to Hemicryptophytes (*Ipomeaaquatica* and *Hydrillaverticillata*) Table -6.

Aquatic weeds are classified according to various habitats which form their eco-environment and become conducive for their growth, reproduction and dissemination. In the present study, some of the identified plants are called as aquatic weeds. They are *Nelumbonucifera, Ipomeaaquatica*(Rooted floating weed), *Cyperusrotundus, Commelinabenghalensis* (emergent weeds), *Cynodondactylon, Hydrillaverticillata, Azollapinnata* (Free floating weeds). These aquatic weeds interface with the static and flow water system. They cause tremendous loss of water from water bodies. Diffuse growth of these weeds provides an ideal habitat for the development of mosquitoes causing malaria and some other diseases. These weeds also serve as vectors for disease causing organisms and can greatly reduce the aesthetic value of water bodies from a recreational point of view. Our study area is enriched with small fishes that are due to the presence of aquatic weeds, because they provide continuous supply of phytoplankton.

The vegetation of the various plant species were classified after Raunkiar's life forms classifications as modified by Ellengberg and Muller-Dombois (1967) and Muller-Dombois and Ellemberg (1974).The form, habitat and nature of cotyledons, life form of occurrence of each species were studied in the field. The biological spectrum for the area has been compared with the Raunkiar;s Spectrum (Raunkiar's 1934, Muller Dombois and Ellemberg 1974).

Comparison of the presently prepared life form spectrum with that of the Raunkiar's normal biological spectrum of world revealed that therophytes were most (67.69%) higher than the normal spectrum and other life forms like phanerophytes (15.38%) and Cryptophytes (6.15%) are higher than the normal spectrum. Chamaephytes (7.69%) and Hemicrypotophytes (3.07%) were found less than the normal spectrum. Therophytes

were recorded five times higher than the normal spectrum and they are the indicators of amount of biotic influence on the vegetation and develop especially in the area where vegetation has been disturbed by overgrazing (Singh and Ambasht, 1975). Barucha and Dave (1994) stressed that higher Therophytes are indicators of the magnitude of influence of man and animals on the habitat Saxena and Singh (1982) prepared the biological spectrum for Himalayan vegetation across different altitudes. According to Asri (2003) therophytes are the indicators of dry conditions and also attributed to human activities. Abd EL-Ghani and Abdel-Khalik (2006) noted that the increase in grazing pressure throughout the southern Mediterranean ecosystems leads to the occupation of the under stories by invasive therophytes and indicates hyperdegradition. Kapoor and Singh (1990) also gave a detailed account of the life forms pattern in the temperature grass lands of Shimla hills, Himachal Pradesh.

The Biological spectrum of study area showed divergence when compared with Raunkiar's normal spectrum depicting the thero-phanerophytic (Therophytes -67.69%; Phanerophytes -15.38%) plant climate of the region. The preponderance of therophytes in the area may be due to the reason that the therophytes are the ephemerals, which survive adverse seasons in the form of seeds and predominantly found in extremes of dry, hot or cold, conditions.

Grasses are widespread than any other family of flowering plants of the world and represented by 10,000 species 261 genera (Karthikeyan, 2005). As in the case of any aquatic ecosystem, monocots dominate the vegetation having more species diversity in contrast to terrestrial habitats. But in this study, dicots are dominant than the monocots. Even though dicots are dominant Poaceae (Grasses), Cyperaceae (sedges), Hydrocharitaceae and Commelinaceae with 2, 7, 3 and 2 species respectively dominated the study area.

Cyperaceae with its wide range of distribution and habit adaptability found a place even in the Pre-Linnaean contribution. An analysis has revealed that most of the species of Cyperaceae belongs to Penninsularindia, while *Kallingabrevifolius, cyperusrotundus, Pycreuspunilis, Maricuscompaitus* etc., are cosmopolitan and *Cyperuscompressus, C. iria, Fimbristylisdichotoma*are pantropical, the rest are more or less restricted in distribution, and show a strong affinity to the flora of Tamil Nadu, India, South east Asia and China. Several species of *Cyperus* and *Fimbristylis* are frequently found as weed and they have a very wide range of distribution in the tropics of India. The present study agrees with the finding of Rao and Varma (1982) that these plants are in the wide range of distribution.

Geographic distribution of terrestrial plant species is often limited by climatic factors, by competition with species that perform better under their local environment and by the reduced reproductive success of range limit populations (Garcia *et al* ., 2000). Moreover high proportion of widely distributed taxa among the aquatic plants may be due to uniformity of the aquatic environment, widespread clonally, high phenotypic plasticity, ecological factors and climate in particular. These factors are known to constrain the distribution of plant species, resulting in large vegetation zones (Walter, 1973). It can be argued that the rest of the species down come from the neighbouring phyto-geographical domains. Jordan (2001) point out that geographical barriers and patterns of long – distance dispersal are often referred to as contributes to the distribution of aquatic flora.

The utilization of aquatic associated macrophytes at a sustainable basis can only succeed if the surface water and aquatic ecosystems are properly managed. Surface water and wetlands, and consequently aquatic plants are constantly threatened by a number of factors which include: drainage of wetlands for crop production, stream channelization and flood control, housing development, solid waste and nutrient loading from domestic sewage and agricultural runoff. The domestic sewage and industrial waste adversely affect the quality of water and consequently the flora and fauna of the water bodies (Verma, 2002). The human activities alter the structure of surface water and give a selective advantage to one, or a few species which develop a large population, 'crowd' out other species and lower the total community productivity.

Aquatic plants are especially sensitive to changes (increases in nutrient concentration and to organic pollutants. The physico-chemical characteristics have been found to exert influences on the biological production in water bodies (Kaushik*et al.,* 2002). The aquatic weeds, which are a common sight in any aquatic system, become a menace for water bodies. In many places de-weeding is a big programme, so in such a situation, if we can harness the potential of the weeds for better

utility in indigenous medicine we can conserve the diversity of macrophytic plant population.

The present study indicates that the studied areas are very rich in flora biodiversity and indigenous knowledge. The local people are dependent on these species not only for domestic uses (especially food, manure, raw-materials), but also to cure various diseases. According to collected information, only small quantities of some species are collected and sell in market. However, there are possibilities to enhance the income of the local communities, if properly managed the habitats and potential species in an integrated manner with the involvement of local people in planning and management of the resources. So it is recommended to initiate the activities, such as inventory of useful species, habitat characteristics, identification of potential species for various economic uses and formulation and implementation of plan of actions taking consideration of the needs of people and sustainable management of the wetlands.

When questioned about the changing status of the existing plants, our respondents mentioned that the alien and invasive species are spreading very fast in the water bodies impacting on the growth of the native species. Some species, especially species of*Nelumbo, Nymphoides* and *Trapa* are declined in abundance during the last decade. Priority should be focused on the conservation of the valuable native species and their habitats with the integrated management measures.

4. CONCLUSION

The present trend of uses of plant diversity in the study area indicated that the uses of plants and traditional practices will continue to play a significant role in the socio-cultural life of these village communities. But the trend of decline of the abundance of some very useful native species, increase of unsustainable anthropogenic practices and encroachments and spreading of invasive species show that action for conservation is urgently needed. Therefore, priority should be given to implement conservation activities with integrated approach for sustainable development.

Table 1. List of aquatic macrophytic species identified in the study area (Kurandikulam, Melasankarankuzhi, Kanyakumari District).

S. No	Botanical Name	Habitat Status
	ANGIOSPERMS DICOTYLEDONS	
	AMARANTHACEAE	
1	*Achyranthesaspera*Linn.	Marginal
2	*Alternantherasessilis*Linn.	Emergent
3	*Amaranthusspinosus*Linn.	Marginal
4	*Digeramuricata*L.	Marginal
5	*Gomphrenacelosioides*Mart.	Marginal
	ASTERACEAE	
6	*Tridaxprocumbens*Linn.	Marginal
7	*Partheniumhysterophorus*Linn.	Marginal
8	*Eclipta alba* Hassk.	Emerrgent
	BORAGINACEAE	
9	*Heliotropiumindicum*Linn.	Marginal
	CONVOLVULACEAE	
10	*Ipomoea aquatica*Forsk.	Emergent
11	*Convolvulus arvensis*Linn.	Margianl
	CAESALPINIACEAE	
12	*Cassia occidentalis*Linn.	Marginal
13	*Cassia tora*Linn.	Marginal
	CAPPARACEAE	
14	*Cleome viscosa*Linn.	Marginal
	EUPHORBIACEAE	
15	*Euphorbia hirta*Linn.	Marginal
16	*Phyllanthus simplex* Rertz.	Marginal
17	*Acalyphaindica*Linn.	Marginal
	LAMIACEAE	
18	*Ocimum sanctum* Linn.	Marginal
19	*Anisomelesmalabarica*Linn.	Marginal
20	*Leucasaspera (*willd.) Spreng.	Marginal
	MALAVACEAE	
21	*Sidarhombifolia*Linn.	Marginal
22	*Abutilon indicum (*Linn*).*	Marginal
	NELUMBONACEAE	
23	*NelumbonuciferaGaertn.* Fruct.	Floating
	NYMPHAEACEAE	
24	*Nymphaeastellata*Willd.	Floating
	LENTIBULARIACEAE	
25	*Utricula*riastellaris*linn.	Submerged
	ONAGRACEAE	
26	*Ludwigiahyssopifolia (*G.Don)	Floating
	PAPAVERACEAE	
27	*Argemonemexicana*Linn.	Marginal
	POLYGONACEAE	
28	*Polygonumgrabrum*Willd.	Emergent
29	*Polygonumbarbatum*Linn.	Emergent
	SOLANACEAE	
30	*Solanumnigrum*Linn.	Marginal
31	*Solanumxanthocarpum*schrad.	Marginal
32	*Datura metal* L.	Marginal
33	*Physalis minima* Linn.	Marginal

	SAPINDACEAE	
34	*Cardiospermumhalicacabum*Linn.	Marginal
	SCORPHULARIACEAE	
35	*Scopariadulcis*linn.	Emergent
	RUBIACEAE	
36	*Lantana camara*Linn.	Marginal
37	*Clerodendrumviscosum*Vent.	Marginal
	MONOCOTYLEDONS-ARACEAE	
38	*Colocasiaesculenta*Linn.	Marginal
	CERATOPHYLLACEAE	
39	*Ceratophyllumdemersum*Linn.	Submerged
	CYPERACEAE	
40	*Maricuscompaitus*Retzius	Emergent
41	*Pycreuspunilis*L.	Emergent
42	*Fimbristylisdichotoma*L	Emergent
43	*Cyperuscompressus*Linn.	Emergent
44	*Cyperusrotundus*Linn.	Emergent
45	*Cyperusiria*Linn.	Emergent
46	*Kallingabrevifolia*Rott ball.	Emergent
	COMMENLINACEAE	
47	*Commelinabenghalensis*Linn.	Emergent
48	*Commenlinanudiflora*Linn.	Emergent
	LEMNACEAE	
49	*Lemna minor* Linn.	Floating
	HYDROCHARITACEAE	
50	*Hydrillaverticillata*Linn.	Submerged
51	*Otteliaalsinoides*Linn.	Submerged
52	*Vallisnariaspiralis*Linn.	Submerged
	PONTEDERIACEAE	
53	*Potamogetonmalaianus*Miquel	Submerged
	POACEAE	
54	*Cynodondactylon*Linn.	Marginal
55	*Chlorisbarbata*L.	Marginal
	THALLOPHYTA (ALGAE)	
	CHAROPHYCEAE	
56	*Charazeylanica*Willd	Submerged
57	*Charanitzii*Schw.	Submerged
58	*Nitella hyaline* Agardh	Submerged
	PTERIDOPHYTA-SALVINACEAE	
59	*Azollapinnata*R. brown	Floating
60	*Salvinianatans*Linn.	Floating
	MARSILEACEAE	
61	*Marsileaquadrifolia*Linn.	Emergent
	SELAGINELLACEAE	
62	*Selaginella*species	Emergent
	MARCHANTIACEAE	
63	*Marchantia*species	Floating
	POLYTRICHACEAE	
64	*Polytrichum commune*	Emergent
65	*Polytrichumjuniperinum*	Emergent

Table 2, Family wise distribution of aquatic macrophytes in the study area (Erattaikulam, Thoopur, Kanyakumari District).

S. No	Family	No. of species	No. of genus	% Composition
1	Amaranthaceae	5	5	7.6%
2	Araceae	1	1	1.5%
3	Asteraceae	3	3	4.5%
4	Boraginaceae	1	1	1.5%
5	Caesalpiniaceae	2	1	3.0%
6	Capparaceae	1	1	1.5%
7	Ceratophyllaceae	1	1	1.5%
8	Charophyceae	3	2	3.0%
9	Commelinaceae	2	1	3.0%
10	Convolvulaceae	2	2	3.0%
11	Cyperaceae	7	5	10.6%
12	Euphorbiaceae	3	3	4.5%
13	Hydrocharitaceae	3	3	4.5%
14	Lamiaceae	3	3	4.5%
14	Lemnaceae	1	1	1.5%
16	Lentibulariaceae	1	1	1.5%
17	Malvaceae	1	1	1.5%
18	Marchantiaceae	1	1	1.5%
19	Marsileaceae	1	1	1.5%
20	Nelumbonaeceae	1	1	1.5%
21	Nymphaeaceae	1	1	1.5%
22	Onagraceae	1	1	1.5%
23	Papaveraceae	1	1	1.5%
24	Poaceae	2	2	3.0%
25	Polygonaceae	2	1	3%
26	Polytrichaceae	2	1	3.0%
27	Pontederiaceae	1	1	1.5%
28	Potamogetonaceae	1	1	1.5%
29	Rubiaceae	2	2	3%
30	Salviniaceae	2	2	3.0%
31	Sapindaceae	1	1	1.5%
32	Scrophulariaceae	1	1	1.5%
33	Selaginellaceae	1	1	1.5%
34	Solanaceae	4	3	6.0%

Table 3. Taxonomic data of aquatic macrophytes of study area.

	D	M	A	P	B	Total
Families	19	9	1	3	2	34
Genera	33	16	2	4	2	62
Species	36	19	3	4	3	65

D – Dicots; M- Monocots; A – Algae; P - Pteridophytes; B- Bryophytes.

Table 4. Habitat status of the identified plants in the area.

Habitats	Marginal	Floating	Emergent	Total
Number of plants	30	7	19	65

Table 5. Identified plants in the study area and its uses

S.No	Name of the plants	Uses
1	*Alternantherasessilis*(L.)DC.	Whole plant used in conditions of kapha and pitta, burning sensation, leprosy, skin disease, dysepesia, haemorrhoids and fever. Leaf used in bone fracture, eye complaints, bite of rabid dog, snakebite and night blind.
2	*Ceratophyllumdemersum*L	Whole plant used as a cooling agent and scorpion sting.
3	*Commelinabenghalensis*L.	The plant is useful to treat bedsores, breast sores and pimples. It is also used to control Haemorrhages.
4	*CynodondactylonPers*	Leaf juice drunk to relieve body pain. Leaf juice mixed with lime applied to cure inflammation.
5	*Cyperusrotundus*L.	Root used in bowl complaints, diuretic, jaundice, sores and wound. Bulb used in dysentery.
6	*Ecliptaprostrata*L.	The whole plant is used to treat jaundice, liver and spleen complaints, malaria, anti-fertility, ulcers and wounds. Roots used as an antidote to snakebite. Plant is squeezed and boiled with coconut oil, applied on the scalp is a good medicine for preventing hair loss and dandruff.
7	*Ipomoea aquatica*forsk	Leaf juice used as a mild purgative and blood purifier.
8	*Ludwigiahyssopifolia*(G.Don).	Leaves used to cure cuts, wounds and sores.
9	*Marsileaquadrifolia*L.	Whole plant is useful in psychopathy, opthalmis, diarrhoea, cough, bronchitis, leprosy, skin diseases, dyspesia, haemmorrhoides, fever and insomnia.
10	*NelumbonuciferaGaetn*	Whole plant is given in hyperdipsia, chloera, diarrhoea, helminthiasis, vomiting and cardiac debility, flowers used as a cardio tonic in fever and diseases of liver. Rhizome used in treatment of piles. Seeds used as cooling medicine for skin diseases.
11	*Nymphaeastellata*wild.	Whole plant used as cardio tonic.
12	*Polygonumglabrum*wild.	Plants used as a febrifuge and the infusion of leaves in colic pain.
13	*Vallisneriaspiralis*L.	Whole plant is used as a stomachic and for leucorrhoea.
14	*Eichhorniacrassipes*(Mart.) Solms	The whole plant used as manure and for fattening pigs.
15	*Hydrillaverticillata*(L.F.)	It is eaten by some fishes and it is a good oxygenator. It is suitable for aquaria.
16	*Otteliaalsinoides*(L.) Pers.	The fruits are eaten by children. The petioles and blades are used as vegetables.
17	*Polygonumbarbatum*L.	The root is used as an astringent and cooling remedy. The leaves and stalks is said to be used as a stimulating wash for ulcers.
18	*Utricula riastellaris*L.	Ecologically the plant is a good oxygenator of water and is used by fishes for food.
19	*Vallisneriaspiralis*L.	Ecologically the plant is a good oxygenator of water and is used by fish for food.

Table 6. Life form classification of the aquatic macrophytic species from the study area.

Life Forms	Name of the Plants	No.of Species	% Composition
Chamaephytes (CH)	*Polygonumglabrum,* P.*barbatum, Charazeylanica,* C.*Nitzii, Nitella hyaline.*	5	7.69
Phanerophytes (P)	*Utricula*riastellaris*, Azollapinnata, Salvinianatans, Ceratophyllumdemersum, Marsileaquadrifolia, Lemna minor, Marchantias*pecies*, Nelumbonucifera, Nymphaeastellata, Ludwigiahyssopifolia.*	10	15.38
Cryptophytes (C)	*Vallisneriaspiralis, Colacasiaesculenta, Potamogetonmalaianus, Otteliaalsinoides.*	4	6.15
Therophytes (T)	*Alternantherasessilis, Eclipta alba, Commelinabenghalensis,* C*. nudiflora, Cyperuscompressus,* C.*rotundus,* C*. iria, Maricuscampaitus, Pycreuspunilis, Kallingabrevifolia, Achyranthusaspera, Amaranthusspinosus, Digeramuricata, Gomphrenacelosioides, Tridaxprocumbens, Partheniumhysterophorus, Heliotropiumindicum, Convolvulus alsinoides, Cassia tora, Cassia occidentalis, Cleome viscosa, Euphorbia hirta, Phyllanthus simplex, Acalyphaindica, Ocimum sanctum, Anisomelesmalabarica, Leucasaspera, Sidarhombifolia, Abutilon indicum, Argemonemexicana, Solanumnigrum,* S*. xanthocarpum, Datura metal, Cardiospermumhalicacabum, Lantana camera, Cleorodendrumviscosum, Cynodondactylon, Chlorisbarbata, Polytrichum commune,* P.*juniperinum, Selaginella*sp*, Physalis minima, Scopariadulcis.*	44	67.69
Hemicryptophytes (H)	*Ipomoea aquatica, Hydrillaverticillata.*	2	3.07

Table 7. Biological spectrum (%) of all life forms found in the study area.

Life Form Classes	No. of Species	Life forms (%) Present study	Raunkiar's normal spectrum and composition (%)	Deviation of Normal Spectrum
Ch	5	7.69	46	-38.31
P^H	10	15.38	9	46.38
C	4	6.15	6	+0.15
H	2	3.07	26	-22.93
Th	44	67.69	13	+54.69
Total	65	100	100	

Ch - Chamaephytes; C - Cryptophytes; H - Hemicryptophytes; Th – Therophytes; P-Phanerophytes

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PHYTOCHEMICAL SCREENING OF *COSTUS MEXICANUS* LIEBM. - AN INSULIN PLANT

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ABSTRACT

Phytochemicals are extensively found at different levels in many medicinal plants. *Costus mxicanus* an important medicinal plant belongs to the family Costaceae. It is used for diabetics by traditional healers. The present study was undertaken to investigate the preliminary phytochemicals in the petroleum ether, acetone and ethanol extracts of leaves, stem and rhizome of *Costus mexicanus* Liebm. and the study revealed the presence of alkaloid, flavanoid, terepenoid in the *Costus mexicanus.*

Keywords: *Costus mexicanus*, preliminary phytochemical**.**

1. INTRODUCTION

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active (bioactive) constituents of plants are: alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes (Edeoga *et. al.*, 2005).

A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Farnsworth, 1966).

Costus is a genus of perennial tropical herbaceous plants from the costus family (Costaceae). *Costus mexicanus* Liebm. is also known as Insulin plant. It is used as a munching supplementary food for the treatment of diabetes. In Mexico, it is used to treat renal disease (Martinez, 1996; Rzedowski, 1979; Caceres *et. al.*, 1987; Argueta *et. al.*, 1994). It is reported to have effects on renal functions and its anti-inflammatory, hypoglycemic actions (Martinez, 1996 and Merina, 2004) and ant diabetic property (Merina, 2005 and Nandhakumar *et. al.*, 2007).The plant is also rich in antioxidants (Shubha, 2010). The plant was reported to contain flavonoids, saponins, reduced sugars and tannins (Comargo *et. al.*, 2006). Hence, this study was designed to undertake the physicochemical and phytochemical screening of various extracts of *Costus mexicanus*.

2. MATERIALS AND METHODS

2.1. Collection and identification of plant materials

The leaves, stem and rhizome of *C.maxicana* were collected from Kannur, Kerala and authenticated by Botanical survey of India, Coimbatore. The voucher specimen of the plant was deposited at the college for further reference.

2.2. Extraction of plant materials

The plant materials (leaves, stem and rhizome of *C.maxicana*) were air-dried at room temperature (26°C) for 2 weeks, after which it was grinded to a uniform powder. The petroleum ether, acetone and ethanol extracts were prepared by soaking 50 g each of the dry powdered plant materials in 300 L of ethanol at room temperature for 48 h. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40°C.

2.3. Physicochemical Analysis:

The coarse powder of leaves, stem and rhizome of *Costus mexicanus* was subjected to various physicochemical studies for determination of ash values and extractive values.

2.3.1. Qualitative screening

The dried, pulverized leaves and roots were subjected to phytochemical analysis to screen for the presence of secondary metabolites such as Alkaloids, Tannins, Saponin, Resins, Flavonoids, Glycosides,

Steroids, Phenols, Terpenoid, Cardiac glycosides and Triterpenoids. The phytochemical screening was carried out using standard procedure (Sofowora, 1993 and Trease and Evans, 2002).

2.3.2. Quantitative screening

2.3.2.1. Alkaloid determination

2.5 g of the powder was extracted using 100 ml of 20% acetic acid in ethanol. The solution was covered for almost 4 hours. Filtrate was concentrated to 25 ml. Concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed (Edeoga *et al*., 2005 and Okwu and Josiah, 2006).

2.3.2.2. Saponin determination

10 g of sample was mixed with 100 ml of 20% aqueous ethanol. The mixture was kept for 4 hours on water bath shaker at 55° C. Filtrate was again extracted in same manner. The combined extract was concentrated to 40 ml over water bath at 90°C. Concentrate obtained was transferred into a separating funnel and 10 ml of diethyl ether was added to it. After shaking vigorously aqueous layer was recovered and ether layer was discarded. The process was repeated. To the aqueous layer n-butanol was added. The whole mixture was washed in separating funnel twice with 10 ml 5% of aqueous NaCl. Upper part was retained and heated in water bath until evaporation. Latter it was dried in oven to a constant weight (Obadoni and Ochuko, 2001; Edeoga *et al*., 2005).

2.3.2.4. Tannins determination

2g of plant powder was extracted thrice in 70% acetone. After centrifuging the sample supernatant was removed. Different aliquots were taken and final volume to 3 ml was adjusted by distilled water. The solution after vortexing were mixed with 1 ml of 0.016M $K_3Fe(CN)_6$, followed by 1 ml of 0.02M $FeCl_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept as such for 15 min. 5 ml of stabilizer (3:1:1 ratio of water, H_3PO_4 and 1% gum arabic) was added followed by revortexing. Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001M gallic acid (Graham, 1992).

2.3.2.5. Carbohydrate determination

0.5 g of plant material was extracted with 80% ethanol. Extract was dissolved in 10 ml water. Different aliquots were prepared and final volume was made to 1 ml by water. 5 ml of 96% of concentrated $H_2SO_4$was added followed by shaking and incubation for 40 min at room temperature. 1 ml of 5% phenol was added to each tube and absorbance was taken at 490nm. Standard curve using different concentrations of 25 mg% glucose (Krishnaveni *et al*., 1984).

2.3.2.6. Proteins determination

1g plant material was extracted using 10 ml water added with few drops of triton X- 100. Supernatant was extracted in acetone and the pellet obtained was dissolved in 0.1 M NaOH. Aliquots were prepared and final volume was made to 1 ml by distilled water. 5 ml of copper reagent was added to tubes, mixed well and incubated for 10 minutes. 1 ml of folin's reagent was mixed. Tubes were incubated for 30 min at room temperature and absorbance was taken at 700 nm. Standard curve was prepared using 50 mg % BSA (Lowry *et al*., 1951).

2.3.2.7. Lipids determination

1g plant sample was dissolved in ether and stirred for a hour. Mixture was centrifuged, supernatant dried and dissolved in ethanol. 0.1 ml of alcohol was taken as blank, olive oil as standard and test sample as unknown respectively. 2 ml of concentrated H_2SO_4 and 5 ml of phosphovanillin reagent was added and mixed well, incubated for 30 min. Absorbance was read at 540nm (Ganai *et al*., 2005).

3. RESULTS AND DISCUSSION

The results of preliminary phytochemical study were tabulated in Table-1. The phytochemical study revealed the presence of steroids, flavonoids, alkaloids, coumarins, triterpenoids, tannins and carbohydrate. The table-2 shows the Quntitative Phytochemical Screening which is measured in g %. The Physicochemical Analysis is described with physical nature, extractive value and ash value in table-3.

Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). Just *et al.* (1998) revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in *C.maxicana* extracts and has supported the usefulness of this plant in managing inflammation. Flavonoids, another constituent of *C.maxicana* leaves, stem and rhizome extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-

angionic, analgesic, anti-allergic, cytostatic and antioxidant properties .The result justifies the use of these plants in traditional medicine for the treatment of various kinds of diseases including infectious disease (Idu *et al.*, 2006).

4. CONCLUSION

This study has shown the scientific basis for some of the therapeutic uses of *C.maxicana* plant in traditional medicine. The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound.

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Table 1: Qulalitative Phytochemical Screening of *Costus Maxicana*

Bioactive Agents	Petroleum ether			Acetone			Ethanol		
	L	S	R	L	S	R	L	S	R
Alkaloid	+	+	+	+	+	+	+	+	+
Flavanoid	+	+	+	+	+	+	+	+	+
Saponin	-	-	+	-	-	-	-	-	+
Glycoside	+	+	-	+	+	-	+	-	-
Tanin	-	-	+	-	-	+	-	-	-
Terpenoid	+	+	+	+	+	+	+	+	+
Resin	+	+	+	+	+	-	-		-
Steroid	+	-	+	+	+	+	+	+	+
Phenol	+	+	-	-	+	-	-	+	-
Cardiac glycoside	+	+	+	-	+	+	-	+	+
Tri-terpenoids	+	-	-	-	-	-	+	-	+

Table 2: Quntitative Phytochemical Screening of *Costus Maxicana*

Bioactive Agents	Quantity/100g of plant material* (i.e. g %)		
Alkaloid	L	S	R
Tannins	1.53 ± 0.02	1.18 ± 0.05	1.73 ± 0.01
Saponins	0.05±0.001	0.03 ± 0.03	0.06 ± 0.003
Flavonoids	0.35±0.0025	0.47 ± 0.04	0.21 ± 0.03
Cardiac Glycosides	1.73±0.036	1.33 ± 0.064	1.47 ± 0.043
Carbohydrates	0.056±0.005	0.067 ± 0.007	0.032 ± 0.003
Lipids	0.375±0.0012	0.474 ± 0.006	0.659 ± 0.004
Proteins	2.44±0.002	2.96 ± 0.003	4.69 ± 0.006

*Results are mean ± SD of triplicate determination on the basis of dry weight.

Table 3: Physicochemical Analysis of *Costus Maxicana*

S.No.	Parameters	Observation		
		Leaves	Stem	Rhizome
I	Physical test			
	Nature	Smooth	Scaly	Scaborous
	Colour	Dark green	Greenish yellow	Brown
	Odour	Odourless	Pungent smell	Pungent smell
II	Extractive value			
	Petroleum ether	8.45	6.82	7.5
	Acetone	9.29	7.34	9.26
	Ethanol	11.86	10.62	9.36
III	Loss of Drying	9.53 %		
IV	Ash Value			
	Total ash	9.2	7.8	8.8
	Acid insoluble ash	2.7	3.9	2.3
	Water soluble ash	3.1	3.6	2.4

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HEPATOPROTECTIVE ACTIVITY OF *ERIA MYSORENSIS* LINDL. (ORCHIDACEAE) PSEUDOBULP AGAINST CARBONTETRACHLORIDE INDUCED TOXICITY

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ABSTRACT

The ethanolic extract of pseudobulb of *Eria mysorensis* at the dose of 100mg/kg body weight (oral) was studied for the hepatoprotective effect using Carbontetrachloride induced liver damage in wistar albino rats. Ethanolic extract showed significant ($p<0.05$) hepatoprotective effect by lowering the serum levels of various biochemical parameters such as serum Glutamic Oxaloacetate Transaminase (SGOT), Serum Glutamic Pyruvates Transaminase (SGPT), Alkaline Phospatase (ALP), Total Bilirubin (TBL), Total Cholesterol (CHL) and by increasing the levels of Total Protein (TPTN) and Albumin (ALB) in the selected model. These biochemical observations were inturn confirmed by histopathological examinations of liver sections and are comparable with the standard hepatoprotective drug Silymarin (100mg/kg body weight) which served as a positive control. The overall experimental results suggests that the biologically active phytoconstituents such as flavonoids, terpenoids, sterols, phenols, saponins and alkaloids present in the ethanolic extract of plant *Eria mysorensis*, may be responsible for the significant hepatoprotective activity and the results justify the use of *Eria mysorensis* as a hepatoprotective agent.

Keywords: Hepatoprotective activity, *Eria mysorensis,* pseudobulp.

1. INTRODUCTION

India has one of the largest tribal population in the world. The forest plays a vital role in the economy as well as daily needs of the tribals. In times of scarcity when the staple food is in short of supply tribals collect many types of wild roots and tubers to supplement their meagre food available at home(Vidyarthi, 1987). Although orchids are being cultivated and valued mainly for ornamental purposes. Some of them are used from time immemorial in traditional practices to treat various medical conditions. More than 13 species of orchids of traditional importance are reported in Kerala forests(Muktesh Kumar, 1987). Orchids, commonly called for the members of Orchidaceae. Normally grow as epiphytes or often as lithophytes or sometimes as saprophytes. It is one of the most diversified plant families of angiosperms comprising of 18,500 species under 788 genera(Mabberley,1997). Mainly distributed in tropical, subtropical to subtemperate regions of the globe. In India, Orchidaceae is the second most diversified family comprising of 184 genera and 1229 species. ***Eria*** is a large genus of orchids with more than 500 species distributed in tropical Asia, Malaysia, Australia, Polynesia and other Pacific islands. It is used for of antidiabetic activity, hepatoprotective activity and aphrodisiac activity. Most of the species have a typical odour of rotting carcasses which attracts the flies to help in their pollination process.

Liver a chief site for intense metabolism and excretion has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailments like hepatitis cirrhosis and alcoholic liver disease (Sharma *et al.,* 1991; Subramaniam and Pushpangadan, 1999). Thus liver diseases are some of the fatal disease in the world today. Therefore, many folk remedies from plant origin are tested for its hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl_4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts (Rubinstein, 1962 and Suja *et al.,* 2002).

2. MATERIALS AND METHODS

Plant material *E. mysorensis* was collected from Velliangiri hills Coimbatore District and is the major hills range of Western Ghats, TamilNadu, India. Collected medicinal plants were identified with the help of field floras (Gamble, 1957; Mathew, 1983; Chandrabose and Nair, 1988). Identification was confirmed at the Botanical survey of India, Southern circle, Coimbatore, TamilNadu.

2.1. Preparation of the extract

Plant material pseudobulb was washed with clean tap water to remove dirty and dried under shade to constant weight for 20 days. The pseudobulb were then cut in to pieces and later grinded to powder using an electrical mill. The powdered material were exhaustively extracted with ethanol for 8hrs using Soxhlet apparatus.The obtained extract was evaporated to dryness at 38°c.

2.2. Animals

Wistar Albino rats weighing 180-240g were used in this evaluation. These rats aged between 2.5 and 3 months were procured from animal house of the laboratory of Agricultural University, Trissur, Kerala. They were housed in well ventilated stainless steel cages at room temperature(24±2 °c) in hygienic condition under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given ad-libitum.

2.3. Experimental design for hepatoprotective activity

The rats were divided into 4groups of 5 rats each. The hepatoprotective activity of the plant extracts was tested using CCl_4 model. Group I(normal control) received neither the plant extract nor CCl_4 for 72 hrs that is they receive food and water only.

2.4. Carbontetrachloride induced hepatotoxicity

Rats were divided into four groups of 5 animals each. The rats of control group (I) received three doses of 5% gumacaciamucilage (1ml/kg, per oral.) at 12 hour intervals (0 hour, 12 hour and 24 hour). The ratsof Carbontetrachloride group (II) received three doses of vehicle at 12 hour intervalsand a single dose of Carbontetrachloride (1.25ml/kg i.p.) diluted in liquid paraffin (1:1)30 minutes after the administration of 1st dose of vehicle. The rats of standard group (III) received three doses of Silymarin (100mg/kg) at0 hour, 12 hour and 24 hour. Carbontetrachloride was administered (1.25ml/kg i.p.) 30 minutes after the first dose of

silymarin. While the rats of test group (IV) received three doses of test extract at the dose of 200mg/kg body weight per oral at 0 hour, 12 hour and 24 hour. Carbontetrachloride was administered (1.25ml/kg i.p.) 30 minutes after the first dose of silymarin test extract (Rao and Mishra, 1997). After 36 hour of administration of Carbontetrachloride, blood was collected and serum was separated and used for determination biochemical parameters.

2.5. Assessment of Hepatoprotective Activity

In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. After 72 hours of drug treatment, the animals were dissected under ether anesthesia. Blood from each rat was withdrawn from carotid artery at the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 minurtes. The separated serum were used for the estimation of some biochemical parameters like Alanine aminotransferase(ALT/SGPT), Aspartate aminotransferase (AST/SGOT), cholesterol, bilirubin and glucose.

3. RESULTS AND DISCUSSION

The effect of ethanol extracts of *E. mysorensis* on initial weight and final weight in normal and liver injured rats were observed. The initial and final body weight, mean weight gain or loss and differences between groups II, III and IV were represented in the table 1. A significant weight loss was observed in the liver injured control group. The body weights were increased in *E. mysorensis* extract treated groups (100, 200,500 mg/kg b.wt.), where as a significant improvement was observed in the group III treated with the standard drug, Silymarin (Fig. 1).

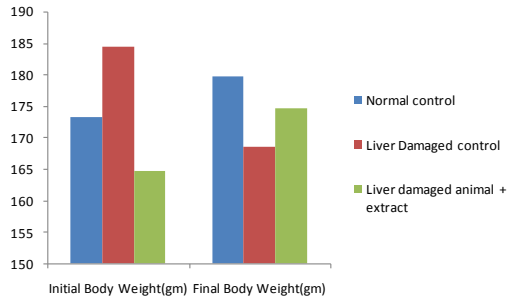
The liver injured rats showed a significant elevation in the total bilirubin, conjucated bilirubin and unconjucated bilirubin when compared to the control group. The administration of ethanol extracts of *E. mysorensis* treated groups at the concentrations of 100, 200, 500mg/kg b.wt showed significant decreases in the levels of total bilirubin, conjucated bilirubin and unconjucated bilirubin when compared to the liver damacied groups. *E. mysorensis* extracts treated groups were significantly comparable to the liver injured rats, received silymarin orally at the dose of 100mg/kg b.wt. (Table- 2 and Fig. 2). This result is compared to the liver injured rats received silymarin orally at the dose of 100mg/kg b.wt. for 14 days. The liver injured group when treated with ethanol extracts of *E. mysorensis* showed elevated levels of all enzymes in group III and IV as compared to the control group.

Estimating the activities of serum marker enzymes, like SGPT, SGOT, ALP can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage (Mitra *et al.,* 1998). The tendency of these enzymes to return to near normally in extract administered group is a clear manifestation of antihepatotoxic effects of the extract.

Table 1. Effect of *E. mysorensis* on the body weight of the Rats Before and After Treatment in the normal, Liver damaged and drug treated rats

Treatment	Dose	Initial Body Weight(gm)	Final Body Weight(gm)	Mean Weight Gain(G)/ Loss(L) (gm)	Percentage Difference
Normal control	0.9%saline	173.24±5.34	179.78±4.88	6.54	3.77
Liver damged control	0.9%saline	184.45±6.67	168.58±5.23	15.87	8.60
Liver damged Animal + *E. mysorensis* extract	100(mg/kg)	182.14±6.62	191.57±5.28	9.43	5.17
	200(mg/kg)	178.34±4.85	189.42±6.58	11.08	6.21
	500(mg/kg)	169.88±4.74	174.38±5.63	4.50	2.64
Standard Drug (Silymarin)	100(mg/kg)	186.96±4.48	196.33±5.78	9.37	5.01

Fig. 1: Effect of *E. mysorensis* on the body weight of the Rats Before and After Treatment in the normal, Liver damaged and drug treated rats.



In accordance with these results, it may be confirmed due to the presence of phytoconstituents such as flavonoids, terpenoids, sterols, phenols, saponins and alkaloids which are present in the ethanolic extract could be considered as, responisible for the significant hepatoprotective activity.

Fig. 2: Effect of *E. mysorensis* extracts on the bilirubin levels (Conjugated and unconjugated) of normal, Liver damaged and drug treated rats.

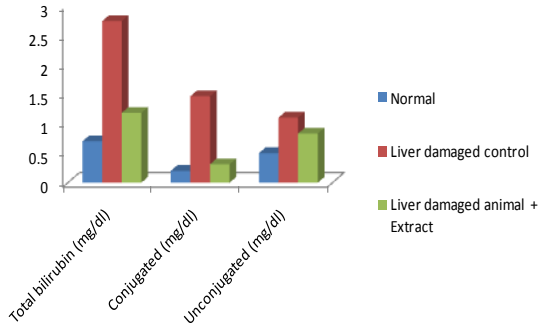


Table 2. Effect of *E. mysorensis* extracts on the bilirubin levels (Conjugated and unconjugated) of normal, Liver damaged and drug treated rats.

Groups	Parameters			
	Dose	Total bilirubin (mg/dl)	Conjugated (mg/dl)	Unconjugated (mg/dl)
Normal	0.9% saline	0.70±0.036	0.20±0.04	0.50±0.03
Liver damaged control	0.9% saline	2.73±0.11	1.46±0.06	1.10±0.05
Liver damaged Animal + *E. mysorensis* extract	100(mg/kg)	1.22±0.03	0.26±0.02	0.96±0.03
	200(mg/kg)	1.02±0.04	0.21±0.04	0.81±0.04
	500(mg/kg)	0.89±0.03	0.19±0.02	0.70±0.06
	100(mg/kg)	0.79±0.06	0.18±0.05	0.61±0.05

4. CONCULSION

In summary, the current study demonstrated that ethanol extract showed a potent protective effect against CCl_4-induced liver injury. Ethanol extract pretreatment significantly inhibited the increase of the serum aminotransferase activities, attenuated oxidative stress-induced mitochondrial dysfunction, and decreased the pathological changes. Thus *E. mysorensis* can be utilized as therapeutics against the liver diseases.

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EVALUATION OF WOUND HEALING ACTIVITY OF METHANOLIC EXTRACT OF *SMILAX WIGHTII* (A. DC.) IN WISTAR ALBINO RATS.

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ABSTRACT

The present experimental study was designed to evaluate the wound healing activity of methanolic extract of *Smilax wightii* A. DC. on incision and excision wound models in Wistar albino rats. The parameters studied were wound breaking strength, wound contraction area, epithelialization period, granulation tissue wet, dry weight and hydroxyproline content in incision wound model, percentage of wound contraction and period of epithelialization in excision wound model. The rats were administered topically with 100mg/kg b.wt. (low dosage), 200mg/kg b.wt. (moderate dosage) and 500mg/kg b.wt. (high dosage) of methanolic extract of *Smilax wightii* (MESW). The activity of the extract treated groups were compared with that of the control 1% Spirit. Framycetin sulphate 0.2% w/w was used as the standard drug. In incision wound model, there was a significant increase in the wound breaking strength in all the experimental groups treated with MESW than that of the control. Similarly, significant ($P<0.001$) decrease in wound contraction area and period of epithelialization were also observed in the test group animals treated with MESW and the standard drug treated groups when compared to that of the control. , a significant increase was observed in granulation tissue wet and dry weight and hydroxyproline content in the test groups treated with MESW compared to the control. In exicision wound model, there was a significant increase ($P<0.01$) in the percentage of wound contraction and decrease in period of epithelialization in the experimental groups treated with 200mg/kgb.wt. (moderate dosage) and 500mg/kgb.wt (high dosage) of MESW. The extract treated groups showed significant improvement in all the wound healing parameters of incision, and excision wound models.

Keywords: *Smilax wightii* extract, wound healing, incision and excision wound models.

1. INTRODUCTION

The genus *Smilax* comprises more than 300 species which are distributed on temperate, tropic and subtropic zones worldwide (Fnaec, 2002). About 24 species of *Smilax* genus are found in India (Saldhana and Wilson, 1976, Ramaswamy, *et al*., 2001). In South India, the genus is represented by 4 species viz., *Smilax zeylanica* Linn., *Smilax aspera* Linn., *Smilax perfoliata* Roxb. and *Smilax wightii* A. DC.(Gamble, 2004). The rare endemic plant, *Smilax wightii* A.DC. is generally distributed in Shola forests at high altitudes in Nilgiri Biosphere Reserve, the Western Ghats, Southern India (Paulsamy *et al*., 2009). The roots of *Smilax wightii* A. DC. have been reported to cure dysentery, amoebiasis, veneral diseases, urinary complaints, fever, antifertility, anaemia, rheumatic-arthritis, veterinary amoebiasis and gastric complaints (Adhikari *et al*., 2010). *Smilax* rhizomes have different kinds of pharmacological behaviours such as antibacterial, antifungal, antioxidant and other activities (Ozoy *et al.*, 2008).

Wounds are physical injuries which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodelling of connective tissue, collagenization and aquisation of wound strength (Suresh Reddy *et al*., 2002). The wound healing process consists of four steps. The first stage is the inflammatory stage which is directed at preventing further loss of blood by platelet accumulation at the site leading to coagulation those results to the formation of thrombus. The debridement stage occurs from the third to the sixth day after injury and involves the appearance of neutrophils to clear contaminating organisms. The proliferation or repair stage is characterized by endothelial budding in the nearby blood vessels forming new capillaries that penetrate and nourish the injured tissue. The maturation stage commences from tenth day to several months depending on wound severity during which the number of capillaries decreases and wound changes from pink or white (Thakare *et al*., 2011).

Some of the synthetic drugs currently used for the treatment of wounds are not only expensive but also cause problems such as allergy, drug resistance etc and this situation has forced scientists to seek alternative drugs (Sai and Babu, 1998). A large number of plants are used by tribal and folklore in many countries for the treatment of wounds and burns. These phytomedicine are not only cheap and affordable but are also safe. The presence of various

life-sustaining constituents in plants has urged scientist to examine these medicinal plants with a view to determine potential wound healing properties (Nayak and Pinto Pereira, 2006).

A survey of literature revealed that no scientific study on the wound healing activity of this plant has been carried out. Hence the present study was undertaken to assess the wound healing activity of methanolic extract of *Smilax wightii* (MESW) on incision and excision wound models in Wistar albino rats.

2. MATERIALS AND METHODS

2.1 *Plant material*

The plant *Smilax wightii* was collected from Uthagamandalam, the Nilgiri Hills, Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist, M. Murugesan, Scientist, SACON, Coimbatore.

2.2 *Preparation of methanolic extract*

500 g of the whole plant powder of *Smilax wightii* was shade dried and used to extract with methanol by using soxhlet apparatus. This extract was stored at 4ºC and used for further studies.

2.3 *Animals*

Wistar albino rats of either sex and of the same age weighing between150 – 250 g were used for the study. They were individually housed, maintained in clean polypropylene cages and fed with commercially pelleted rat chow (M/s Hindustan Lever Ltd. Mumbai) and Water *ad libitum*. The experimental protocol was subjected to the scrutiny of Institutional Animal Ethical Committee for experimental clearance (Reg No. 659/02/a/ CPCSEA).

2.4 *Toxicity studies*

The acute toxicity studies were carried out in adult male Wistar Albino rats weighing 180-250g. The animals were fasted overnight and 100 -1000 mg/kgb.wt of the test extract was given to various groups containing 6 animals in each group. The treated animals were monitored for 14 days for their behaviour, general health and mortality.

2.5 *Experiment*

The surgical interventions in incision and excision wound models were carried out under sterile conditions using ketamine anaesthesia (120mg/kgb.wt). The experimental animals were divided in to five groups of six animals in each group and received the following treatments. The first group were treated with 1% spirit topically and considered as untreated control. The second group were treated with 100mg/kgb.wt (low dosage) of MESW, the third group with 200mg/kgb.wt (moderate dosage) of MESW, the fourth group with 500mg/kg b.wt(high dosage) of MESW and the fifth group were treated with 0.2% w/w Framycetin Sulphate (FSC) topically (standard drug). The MESW was applied on the wound for the test groups from the first day till the day of healing. The level of dosage was 2ml for all the three test groups.

2.6 *Incision wound*

The rats were anaesthetized prior to and during creation of the wound. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, six centimeters in length was made through the skin and cutaneous muscle on the back (Ehrlich and Hunt, 1968). After the incision, surgical sutures were applied to the parted skin at intervals of one centimetre. The wounds were left undressed. The sutures were removed on the 8th post wound day and the treatment was continued. The wound-breaking strength was measured on the 10th day by the method of (Lee, 1968).

2.7 *Determination of wound breaking strength*

The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposite to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wounded edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound, and the procedure was repeated on the contra lateral wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group.

2.8 *Determination of granulation tissue dry weight and wet weight*

In the healed tissues excised from incision wound model rats, the wet weight of the granulation tissue was noted. These granulation tissues were dried at 60°C for 12 hours, and weighed, and the dry weight was recorded. To the dried tissue added 5 ml 6 N HCl and kept at 110°C for 24 hours. The neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline (Neuman and Logan, 1950).

2.9 *Estimation of Hydroxyproline*

Hydroxyproline present in the acid hydrolysate of granulation tissue oxidized by sodium peroxide in the presence of copper sulfate, when complexed with para-dimethylaminobenzaldehyde, develops a pink color that was measured at 540 nm using colorimetry.

2.10 *Excision wound model*

Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by (Morton and Malon,1972). The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area 500mm^2 and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade or pointed scissors. The entire wound was left open (Diwan and Tilloo,1982). The wound closure rate was assessed by tracing the wound on days 2, 4, 6, 8, 10, 12 and 14 post-wounding using transparency papers and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar without any residual raw wound gave the period of epithelialization.

2.11 *Statistical analysis*

The data is expressed as mean ± SEM and subjected to one way ANOVA and the level of significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

No sign of toxicity was noticed on the behaviour and general health of the animals when exposed to the extract at a dosage of 100-1000mg/kgb.wt. No deaths were also observed.

The wound contraction area and the period of epithelialization were observed at an interval of four days till the sixteenth day of wound healing. A rapid decrease in both the parameters were observed in the experimental groups treated with (MESW) and also in framycetin sulphate treated groups when compared to that of the control group in incision wound model (Table-2).

The granulation tissue wet and dry weight and the hydroxyproline content showed significant ($P<0.01$) increase in the extract treated groups when compared to the control. (Table-3). The increase in dry granulation tissue weight in the test group animals suggests higher protein content. The methanolic extract of *Smilax wightii* demonstrated a significant increase in the hydroxyproline content of the granulation tissue indicating increased collagen turnover. Collagen, the major component which strengthens and supports extra cellular tissue is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen (Kumar *et al*., 2006).

The wound contraction was assessed at an interval of two days till the fourteenth day of wound healing. It was observed that the percentage of wound contraction increased and the epithelialization period decreased significantly in excision wound model with the application of MESW as compared to the control group. The standard drug framycetin sulphate also showed profound activity in the percentage of wound contraction in excision wound model rats (Table-4). Preliminary phytochemical screening revealed the presence of tannins, alkaloids, flavonoids, terpenoids, phenols and tannins in *Smilax wightii*. Flavonoids have been documented to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing (Devipriya and Shyamladevi, 1999).

4. CONCLUSION

The results of the present study has led to the conclusion that the methanolic extract of *Smilax wightii* has exhibited prominent wound healing activity in incision and excision wound models with significant results in all the parameters studied.

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Table 1. Effect of methanolic extract of *Smilax wightii* (MESW) on wound breaking strength in incision wound model.

Groups (n)	Wound breaking strength(g)
Control - I	318.26± 4.18
MESW-100mg/kg (low) - II	431.16± 4.84 **
MESW-200mg/kg - (moderate) - III	482.16 ±3.93 **
MESW-500mg/kg (high) - IV	514.1±3.84 ***
Standard drug (FSC) - V	365.16 ±3.54

* P < 0.05,** P<0.01,*** P<0.001, compared to control vs drug treated groups values are mean ± S.E.M. (n = 6)

In incision wound model, the extract treated animal (group II, IIIand IV) showed significant improvement in wound breaking strength when compared to the control group (Table-1).

Table 2. Effect of methanolic extract of *Smilax wightii* (MESW) on incision wound model. mean ± SE wound contraction area (mm²)

Groups (n)	4th day	8th day	12th day	16th day	Epithelization period
Group I	381.16±1.48	361.16±2.91	210.16±2.93	138.13±2.54	26.16±3.13
Group II	284.16±2.16	232.13±1.93	156.84±2.06**	30.16±1.22***	17.84±0.54***
Group III	230.16±1.84	176.84±2.04*	76.16±0.93***	22.15±1.16***	16.34±0.84***
Group IV	216.84±3.16	184.92±3.10**	31.18±0.93***	1.16±0.09***	12.28±0.85***
Group V	211.16±3.11	152.84±1.93***	54.13±0.18***	08.16±0.34***	11.65±1.13***

* P < 0.05,** P<0.01,*** P<0.001, compared to control vs drug treated groups values are mean ± S.E.M. (n = 6)

Table 3. Effect of methanolic extract of *Smilax wightii* (MESW) on granulation tissue wet, dry weight and hydroxyproline content in incision wound model.

Groups (n)	Granulation tissue wet weight (mg)	Granulation tissue dry weight (mg)	Hydroxyproline (mg/tissue)
Control - I	82.6 ±2.16	12.5± 0.91	32.4 ±1.90
MESW-100mg/kg (low) - II	131.58± 1.93**	21.13± 0.16 **	80.2± 4.16*
MESW-200mg/kg - (moderate) - III	172.46 ±3.84**	22.5± 0.34 **	*85.84± 2.27***
MESW-500mg/kg (high) - IV	188.54± 4.20 **	24.16± 0.93**	95.96± 2.55**
Standard drug (FSC) - V	124.16 ±3.40*	18.4± 0.16**	92.16± 2.88*

* P < 0.05,** P<0.01,*** P<0.001, compared to control vs drug treated groups values are mean ± S.E.M. (n = 6)

Table 4. Effect of methanolic extract of *Smilax wightii* (MESW) on percentage of wound contraction in excision wound model.

Groups	Epithelization Period (days)	Excision Wound model% of Wound contraction in different days						
		2	4	6	8	10	12	14
Group I	20.16±0.28	16.31±0.27	28.16±0.16	34.65±0.78	41.16±0.75	55.16±0.93	63.16±0.91	69.54±1.16
Group II	13.42±0.84**	21.34±1.06	43.91±0.84*	59.16±0.93	76.28±0.16*	84.24±0.16**	87.16±1.84	93.16±0.84**
Group III	15.13±0.92**	29.16±0.84*	58.31±0.96*	68.36±0.91*	82.68±1.16**	96.1±1.34*	98.54±0.91**	99.13±1.06**
Group IV	17.83±0.28*	30.84±0.91*	62.84±0.84*	75.16±10.6*	89.16±0.28*	90.33±1.81*	96.84±0.56**	98.84±0.16**
Group V	16.33±0.27	24.13±0.18*	60.33±0.16*	69.26±1.06*	85.16±0.18*	91.36±0.91*	94.32±0.84**	97.29±1.04**

* P < 0.05,** P<0.01,*** P<0.001, compared to control vs drug treated groups Values are mean ± S.E.M. (n = 6)

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PURIFICATION OF TOXIC DETERMINANT LECITHINASE FROM *XENORHABDUS* SP. AND THEIR LARVICIDAL POTENTIAL

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ABSTRACT

A symbiotic bacteria *Xenorhabdus* sp. was isolated from Entomopathogenic nematode *Steinernema* sp. using Insect bait method. This resulted in primary and secondary colony production. The primary colonies were selected for the lecithinase assay. *Xenorhabdus* produce various Exoenzymes in which the present study was concentrated on the exoenzyme Lecithinase which is the toxic determinant. Lecithinase activity of the bacteria was confirmed by lecithinase assay by spot inoculation of bacterial isolates on the nutrient broth supplemented with egg yolk emulsion, the opalescent zones were observed around the colonies producing the lecithinase enzyme after 24 hrs and lecithovitellin reaction was also performed by inoculating the bacterial isolates into the egg yolk broth which resulted in the formation of opalescent suspension and flocculation of particles floating in the media. Lecithinase enzyme was partially purified using Sephadex G-200 column chromatography. Protein profiling of partially purified lecithinase enzyme showed a single band at 70kDa. Larvicidal activity of lecithinase was tested on Coleopteran white grub (*Phyllophaga* sp).It was found that with increasing concentrations of lecithinase, there was increase in mortality of the pest and vector. Thus this study confirms that the toxic determinant lecithinase can be used as potent larvicides for the control of insect pests of plants. The development of new strategies including naturally occurring larvicides to control white grub (*Phyllophaga* sp.) and it is important as the chemical larvicides may harm other soil dwelling organisms as well as humans.

Key word: *Xenorhabdus* sp., Lecithinase, larvicidal potential.

1. INTRODUCTION

Nematodes are round worms which are free living or parasites. Many of the parasite species cause diseases in plants, animals and others are beneficial in attacking insect pests are Entomopathogenic nematodes.Entomopathogenic nematodes (EPNs) in the families *Steinernematidae* and *Heterorhabditidae* represented by the genera *Steinernema* and *Heterorhabditis* respectively are lethal to insect parasites. They are symbiotically associated with mutualistic bacteria of genus *Xenorhabdus* for *Steinernematidae* and *Photorhabdus* for *Heterorhabditis* (Akhrust, 1993). Thus, it is nematode/bacteria complex that works together in biological control of several important insect pests (Kaya and Gaugler, 1993, Grewal and Georgis, 1998) and are important biotic factors in suppression of insect pest populations in soil. The virulent species of these nematodes are commercially produced as biological control agents all over the world encompassing North America, Europe, Asia and Australia in glasshouse crops, orchards, ornamentals, turf, lawn, and forestry. India has a great potential to exploit these beneficial nematodes to suppress the pest species.

The infective juvenile (IJ's) of EPN is a microscopic organism having 0.5 to 1.5 mm long depending on species. The third stage juvenile of these nematodes have closed mouth and anus and cannot feed until it finds an insect. It enters through the natural body openings (mouth, anus, spiracles) or thin cuticle and penetrates into the hemolymph using mechanical and enzymatic means (Abu hatab *et al*., 1995) and release bacteria.

Xenorhabdus sp. are gram negative, motile, rod shaped, faculatively anaerobic entomopathogenic bacteria belonging to family *Enterobacteriaceae* which are symbiotically associated with the infective stages of Entomopathogenic nematode *Steinernema* sp belonging to the families *Steinernematidae*. This bacteria nematode association is highly toxic to many insects and pests species and in most cases bacteria alone is more virulent once they are circulated with hemolymph of the pest (Forst and Nealson, 1996).

Xenorhabdus sp. secretes an array of exoenzymes that stimulate macro molecular degradation; the products together with bacteria themselves provide a nutrient for nematode growth and reproduction. When nematode population increases, nutrients become limiting in the insect cadaver, hence the nematode progeny re-associate with bacteria and differentiate into colonized, non-

feeding IJs (Infective Juveniles) that emerge into the soil to forage for new hosts.

Lecithinase or phospholipases are one of the extracellular enzymes released by bacteria that have the ability to destroy animal tissues by destroying lecithin (phosphatidylcholine) in cell membranes. Bacterial lecithinase hydrolyses this lecithin which is charged so that it is soluble in water, whereas the compound formed by the hydrolysis is diglycerides which is not charged and insoluble in water.

2. MATERIALS AND METHODS

2.1.Isolation of Symbiotic bacteria Xenorhabdus sp. from entomopathogenic nematode

The Symbiotic bacteria *Xenorhabdus* sp. were isolated from the haemolymph of the entomopathogenic nematode infected larvae Rice moth (Corcyra cephalonica). Nematode infected cadaver were collected from the Insect Baiting trap and were surface sterilized. The larvae were cut open for the collection of haemolymph. This collected haemolymph were streaked on the freshly prepared NBTA media (NA + 0.00 25% bromophenol blue + 0.00 4% triphenyltetrazolium chloride) (Akhurst, 1980). Primary colonies and secondary colonies were identified based upon the uptake of bromophenol blue from the NBTA media. Primary forms of the bacteria were maintained each week by streaking on to the fresh media and used for further studies. Morphological and biochemical identification of bacteria was performed.

2.2. Lecithinase activity of bacteria Xenorhabdus sp.

The ability of bacteria to produce the lecithinase enzyme was determined by this method. A loop full of 24 hrs grown culture was taken and spot inoculated on nutrient media supplemented with egg yolk emulsion (10 ml egg yolk in 15ml sterile distilled water). The plates were incubated at 28°C for 24 hrs. Degradation of lecithin present in the egg yolk resulted in the formation of opaque precipitation around the colony. Lecithinase activity as also determined by Lecithovetlin reaction by inoculating the bacterial isolates in nutrient broth supplemented with egg yolk emulsion and incubated at 28°C for 48 hrs.

2.3. Bacterial Lecithinase preparation

Pure primary colonies of *Xenorhabdus* sp. were harvested and inoculated in 250ml conical flask containing 50 ml of nutrient broth and incubated in a shaker incubator at 25°C for 6 days. Cell free supernatant was collected by centrifugation at 10000 rpm for 30 min at 4°C. Proteins present in the cell free supernatant were precipitated by 12% trichloroacetic acid. The precipitated proteins were dialysed over night at 4°C against 1mM Tris HCl buffer and the dialysed samples were used for partial purification by Sephadex G-200. The active fractions were pooled together and used for the determination of enzyme properties like pH, temperature, stability and molecular weight determination of concentration of purified protein sample was determined by Lowry's method.

Agar diffusion assay (Giskow *et al.,* 1988) was performed with 1% agar gel containing 0.01% lecithin, 0.1M NaCl and 0.02 Tris (pH 9). 3µl of crude preparation were added to wells made in the gels and incubated for 24 hours, enzymatic activity was determined by measuring the radius (in millimeters) of the precipitation zone in each gel.

Protein profiling of lecithinase was done using SDS-PAGE. (Laemmli, 1970). Samples were purified using crude dialysis and the purified samples were loaded on to the gel.

2.4. Larvicidal activity of Lecithinase against White Grub (*Phyllophaga* sp.)

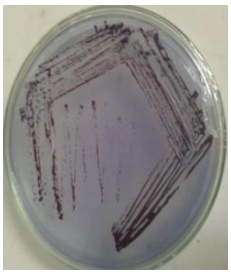
Third instar larvae of white grub (larva of June beetle) were collected from the field of Kodappamund, Ooty,White grub (*Phyllophaga* sp.) 100g of fine sterilized soil was put into plastic bottles and the moisture content were adjusted to 10% by adding requistite amount of sterilized distilled water. 25 white grub were placed in each bottles. The larvae were then sprayed with 2ml of lecithinase (in mg) by a hand sprayer. Each treatment was repeated thrice. All the bottles were sealed with parafilm and incubated at 25°C for 48 hrs. Water alone was sprayed as control. The bottles were examined daily for the mortality.The lethal concentration (LC_{50}) Lecithinase of were calculated using MS Excel 2007 package.

3. RESULTS

Xenorhabdus isolates were found to be Gram positive, rod shaped, had ability to uptake Bromophenol blue from NBTA media (Fig1 A) and neutral red dye from Mac Conkey media (Fig1 B) and biochemical characterization showed negative for Catalase, Oxidase, indole, urease, Lactose fermentation, Glycerol fermentation and showed positive for gelatin hydrolysis, Nitrogen reduction test, Maltose fermentation, Methyl red (Figure 1).

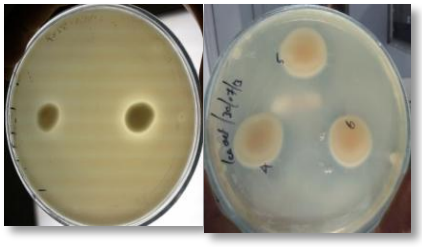
Figure 1: Biochemical and Morphological Characterization of bacterial isolates of *Xenorhabdus* sp.

(+) Positive, (-) Negative, *Xhd- Xenorhabdus* isolates.

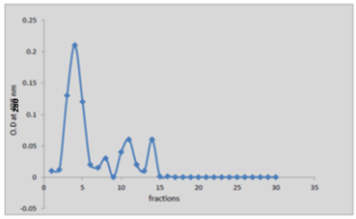
(a)  (b) 

(a) Uptake of Bromophenol blue from NBTA media; **(b)** Uptake of Neutral red dye from Mac Conkey media.

Lecithinase activity of bacteria were positive showing opalescent zone around the colonies producing the lecithinase enzyme whereas, in liquid broth showed opalescent suspension and flocculation of particles floating in the media as a curd. This confirms that the isolated symbiotic bacteria *Xenorhabdus* sp. produces the lecithinase enzyme. Lecithinase enzyme was extracted by inoculating the bacteria *Xenorhabdus* sp. in nutrient broth. Crude preparation of lecithinase enzyme was precipitated with 12% Trichloroacetic acid and purified using Sephadex G-200 Column Chromatography. Protein content for all the fractions were determined at 280 nm by UV-Spectrophotometer. The peak fractions were assayed for observing the presence of lecithinase enzyme in the fractions (Fig 4).This fraction was used for checking the Larvicidal potential.



Lecithinase activity of Bacteria *Xenorhabdus* isolates on Nutrient media supplemented with egg yolk emulsion



Column purification (Sephadex G-200) of lecithinase enzyme in *Xenorhabdus* sp.

3.1.Characterization of lecithinase enzyme

3.1.1 Effect of Temperature on the activity of the enzyme Lecithinase

The optimum temperature of the enzyme lecithinase, in various temperature ranging from 10°C-70°C The optimum temperature activity was noticed at 30°C, after which the activity of the enzyme was found to decrease. (Fig 6).

3.1.2. Effect of pH on the activity of the enzyme Lecithinase

The activity of the enzyme lecithinase in the presence of different pH ranging from 3.0 to 10 using the phosphate buffer was observed. The enzyme exhibited optimum activity at the pH 7.0. (Fig 7).

3.2 Effect of Temperature on the Stability of the enzyme Lecithinase

Lecithinase was treated at different temperatures ranging from 10°C-70°C and the stability of the enzyme was observed. The enzyme was found to be stable upon 40°C To 50 °C and therefore the enzyme activity was found decreasing below 40°C (Fig 8).

3.3. Larvicidal potential of the lecithinase against White grub (*Phyllophaga* sp.)

Larvicidal potential of the toxic determinant lecithinase was performed against white grub (*Phyllophaga* sp.). Lethal Concentration (LC_{50}) of Lecithinase against White grub (*Phyllophaga* sp.) was found to be 8.6 mg. Linear regression analysis resulted in a value of 0.969 which was found to be significant at $p<0.01$.

Fig 6 Effect of temperature on the activity of the lecithinase enzyme of *Xenorhabdus* sp.

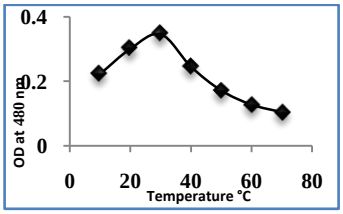


Fig 7: Effect of pH on the activity of the lecithinase enzyme of *Xenorhabdus* sp.

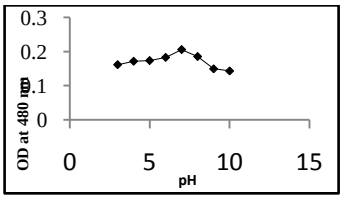
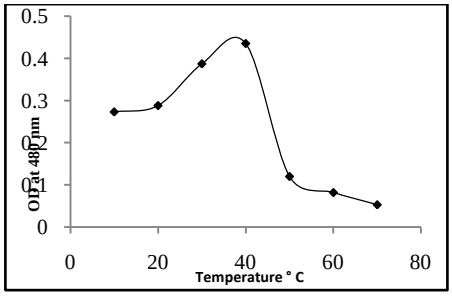


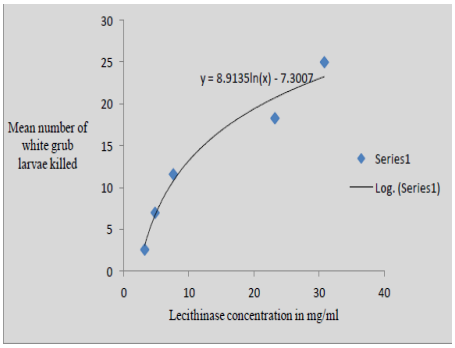
Fig-3 Effect of temperature on the stability of the lecithinase enzyme of *Xenorhabdus* sp.



Lecithinase concentration and percentage of Mortality

S.No	Bacterial colonies	CFU* 10^{-7}/ml	Mean of dead larvae	% of Mortality
1.	154	1.5	1	4
2.	175	1.7	2	8
3.	282	2.8	6.6	26.4
4.	362	3.6	12.6	50.4
5.	456	4.5	25	100

Lecithinase LC_{50} determination for White Grub (*Phyllophaga* sp.)



4. DISCUSSION

Xenorhabdus sp is a gram-negative bacterium belonging to family *Enterobacteriaceae*. The bacteria reside as endosymbionts in the foreguts of soil nematodes belonging to the genus *Steinernema*. Bacteria are released from the gut upon invasion of the insect hemocoel by the nematode. Bacterial multiplication and secretion of toxic proteins are the primary causes of death of the insect host. The bacteria alone are known to be sufficient to cause larval mortality following injection into the hemocoel or following oral administration when they are mixed in the diet. (Khandelwal and Bhatnagar, 2003)

In the present study, the hemolymph of the insect *Corcyra cephalonica* larvae infected with five isolates of different Entomopathogenic nematodes were streaked on NBTA media for the isolation of the symbiotic bacteria *Xenorhabdus sp* .The infected rice moth (*Corcyra cephalonica)* were brown in colour obtained from five soil samples, which was in accordance with the finding of Woodring and kaya (1988).

The mutualistic mode of living of the entomopathogenic bacterium in the nematode gut turns into a pathogenic mode when the bacterium enters the larval hemocoel. *X. nematophilus* is known to secrete highly potent protein toxins into the hemocoel, which rapidly kill the larval host. The insect carcass provides a rich nutrient source on which both the bacteria and the nematode feed, grow, and replicate(Khandelwal and Bhatnagar, 2003).

In this study the cultures were found to be negative for indole production, voges proskaures test, catalase, lactose fermentation and urease and showed positive results for maltose fermentation, glycerol fermentation, citrate utilization, nitrogen reduction test and gelatin hydrolysis test. These results were similar to the reports of Boemare and Akhrust (1998). Morphological characteristics of the symbiotic bacteria used for the present study showed that it was gram negative, rod shaped, motile and have ability to absorb bromophenol blue from NBTA media and neutral red dye from Mac Conkey media. These results were also observed by Boemare and Akhrust (1988) and Yamanaka et al. (1992). This confirmed that the bacteria was *Xenorhabdus* sp.

Xenorhabdus sp. selected for the present study showed lecithinase activity**.** Lecithinase activity of the *Xenorhabdus* isolates was observed by the formation of an opalescent suspension which raised to the top of the medium as a curd. This was based on Macfarlane and Knight (1941) method, who found that the change in the egg yolk broth is due to the hydrolysis of lecithin. The enzymatic hydrolysis of lecithin results in the loss of its emulsifying properties and causes the separation of fatty substances in the egg yolk media.

Xenorhabdus sp are known to produce lecithinase along with other enzyme like protease, lipase which is involved in providing the nutrients for both the nematode and the bacteria. These bacteria release digestive enzymes into the haemolymph of the insect cadaver and breakdown the macromolecules of the insect cadaver to provide nutrient supply (Frost and Nealson, 1996). It has been speculated that it contributes to the

inactivation of the insect immune system (Issacson and Webster, 2002).

In the present study, the Lecithinase activity for the crude and the purified fraction by sephadex columns was observed by agar diffusion method. Crude and the 4th fraction (Sepdadex G- 200) showed the precipitation zone around the wells in Agar diffusion method.

Giskow et al. (1988) and Thaler et al. (1998) found that the lecithinase purified from *Xenorhabdus nematophilla* only in 6th and 8th fractions using HPLC showed the total activity of lecithinase in agar diffusion method. Thaler et al. (1998) found that only the phase I variants of *Xenorhabdus nematophilus* and *Xenorhabdus bovienii* strains produced lecithinase activity when the bacteria were grown on a solid lecithin medium. Singh et al. (1999) observed that the lecithinase activity in *K. pneumoniae* produced lecithinase positive colonies on egg yolk agar. Hence in the present study it was confirmed that the bacteria *Xenorhabdus* sp from primary colonies produced lecithinase enzyme.

The partially column purified enzyme was subjected to optimization studies. In present study, the maximum activity of the enzyme was found at 30°C and pH 7.0 and was stable at the temperature 40°C.Change in pH and the temperature effects the enzyme activity. pH and temperature may change the properties of the substrate so that it cannot bind to the active sate or it cannot undergo catalysis. So enzyme typically have an optimum activity with respect to pH and temperature conditions.

In the present study, a single band was seen on the SDS-PAGE from purified fraction using Sephadex G -200 column indicating the lecithinase in the culture supernatant of *Xenorhabdus* sp. had a molecular mass approximately 70KDa. This was also confirmed by zymogram assay which showed a single very faint and thin precipitated band.

Singh et al. (1999) noted that Lecithinase activity in *Klebsiella* was a rare trait as out of 208 strains of *Klebsiella* belonging to 3 species only 4 strains of K. *pneumoniae* produced lecithinase positive colonies on egg-yolk-agar. Purified lecithinase was determined to be a high molecular weight (70 kDa), crystalizable, anionic (pI, 3.5) protein. It possessed cytolytic, hemolytic and dermonecrotic activities but did not induce fluid accumulation in rabbit ileal loop or infant mouse guts.

An important alternative measure to chemical insecticides is biological control measure which involves the regulation of pest population using natural control agents such as predators, nematodes and microbial insecticides. It is the use of one biological organism to control another, releasing beneficial bacteria, fungi or arthropods to limit pest infestation .

The *Xenorhabdus* lecithinase may participate in the virulence of the nematobacterial complex by allowing intracellular bacterial growth in insects in the same way that phospholipase C acts in *B. thuringiensis* virulence (Bucher, 1960). Many of these lecithinases have been purified and characterized as single secreted-polypeptide proteins. Lecithinases are toxic determinants, as well as a means of securing bacterial supplies of phosphates. They may also have an important role in the induction of pathogenicity in host organisms (Thaler *et al., 1998*).

5. CONCLUSION

This study thus proves the bioactive potential purified enzymes like lecithinase from the entomopathogenic and symbiotic bacteria *Xenorhabdus* sp have Larvicidal activity against the white grub (*Phyllophaga* sp.). It also offers scope for discovery of many more such products from the bacteria associated nematodes which could be used to control insect pests.

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STUDIES ON THE PRESENT STATUS OF ENDANGERED NILGIRI TAHR (*HEMITRAGUS HYLOCRIUS*) IN MUKURTHI NATIONAL PARK, NILGIRIS, TAMILNADU, INDIA

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ABSTRACT

The Nilgiri tahr is found mainly in the Nilgiris, Anaimalai's and then southwards at elevations of 4000-8000 feet (Prater, 1965). Nilgiri tahr (*Hemitragus hylocrius*) belongs to the family Bovidae. Uncontrolled hunting and conversion of habitat to plantations and human related pressure have resulted in the decline of the population (Schaller, 1977). This study was undertaken to determine the present population status, structure and distribution of the Nilgiri tahr in the Nilgiris. Each sector was enumerated on to successive days. Population pattern was studied by foot survey. A census was carried out with forest Department. In addition overall population of Nilgiri tahr within the park is decline due to biotic, abiotic and manmade activities. Grouping pattern of Nilgiri tahr primarily based on seasonal influence and individual fitness. It indicated that more Nilgiri tahr in north sector of the park but historically the south sector contains high density. The estimates suggest that predator accounts for almost all mortality experienced by Nilgiri tahr.

Keywords: *Hemitragus hylocrius,* Nilgiri tahr, Mukurthi National Park.

1. INTRODUCTION

Nilgiri tahr (*Hemitragus hylocrius*) belongs to the family Bovidae and subfamily Caprinae. The Nilgiri tahr in the same tribe as aoudad, bharal, goats and sheep. The Nilgiri tahr was first named Kemas hylocrius by Ogilby in 1838 (Lydekker, 1913). Warryatto is the rendition of the local Tamil name of the Nilgiri tahr "Varrai addu". In 1959, Blyth included the Nilgiri tahr in the genus Hemitragus, naming it *Hemitragus hylocrius* (Lydekker, 1913) the name that persists to date.

The Nilgiri tahr is found mainly in the Nilgiris, Anaimalai's and then southwards at elevations of 4000-8000 feet (Prater, 1965). The present range is restricted along a narrow stretch of 400km between Nilgiri hills in the north and Ashambu hills in the south (1130°N'-8°20'N). It habitat has been reduced to less than one tenth of the local range of the species in the past (Schaller, 1977). The Nilgiri Wildlife Association conducted the first census of the tahr in 1963. The tahr actually seen and counted amounted to 292. Finally in 1997 had a census and recorded only 147. Uncontrolled hunting and conversion of habitat to plantations and human related pressure have resulted in the decline of the population (Schaller, 1977; Davidar, 1978).

2. MATERIALS AND METHODS

Endangered Nilgiri tahr (*Hemitragus hylocrius*) is endemic to the Western Ghats Mountains. Studies on population status of Nilgiri tahr at Mukurthi National Park, Nilgiris, Tamilnadu was conducted for the period of 2007 – 2008. The endangered Nilgiri tahr (*Hemitragus hylocrius*) is endemic to the Western Ghats Mountains in Tamilnadu and Kerala Anaimalai's Conservation unit, Eravikulam National Park and Nilgiri hills at Mukurthi National park consisting viable population of tahr.

2.1. Historical abundance and distribution

To determine the historical abundance and distribution of Nilgiri tahr (*Hemitragus hylocrius*) in the Western Ghats of the Nilgiri plateau, we reviewed published papers, reports of surveys and hunters accounts. In addition we interviewed naturalists, hunters and shikaris (game watchers) who frequented this area since 1960's.

2.2. Present abundance and distribution

To determine the present status of Nilgiri tahr (*Hemitragus hylocrius*) in the Mukurthi National park we conducted foot survey, oral interviews, census and total counts. When we sighted tahr we classified them into sex and age classes based on body size, pelage colour and horn size. We used the six classes described by Schaller, 1971, with modifications and details from Rice, 1984. However, while computing the mortality rate for the young age class we estimated the number of young by assuming that 90% of the adult female gave birth each year

(Schaller, 1977; Rice, 1988). We used this estimate as to record individual birth during this study and probably overlooked many very young tahr.

2.2.1. Foot survey

We used foot survey primarily to obtain the best estimate of the population size. For the foot survey we divided the 78km^2 park into 5 sectors. The first sector included Pandiar top, Nilgiri peak and Devabetta. The second sector included Peechakal Bettu, Peechal Bettu, Chinna Mukurthi and Mukurthi Peak. The third sector included Western catchment III (WC III), Western catchment II (WC II) and Chattiparai. The fourth sector included Western catchment I (WC I) and Bangitapal. The fifth sector included Nadugani, Sispara and Kinkergundi. We attempted to visit each sector once every two months however.

The study was conducted in Mukurthi National Park and we used 'recky walks' method at 0.8 km h-1 on both pre-existing and new routes, with a pedometer used to record distance walked. During 33 and 42 days of walks in the study area the average distance covered per day were 8.2 km and 7.5 km respectively. The total distances walked during day time were 264 km and 336 km in Mukurthi National park. Additional information on the presence and absence of mammal's species was gathered from forest staff, local inhabitants, foot prints, faecal deposits, calls, kills, foraging and roosting signs.

2.2.2. Oral interviews

During the study Tamil Nadu forest department personnel and other frequent visitors to the park, such as fuel wood extraction laborers, tourist guides and naturalist to report all sighting of tahr.

2.2.3. Census

A census covered all parts of the park simultaneously and we divided the park and its surrounding area in to 16 sectors. Crews of three or four members, including forest department employees who has familiar with the area surveyed each sector. All participants of the census were trained to use compass and map to identify mammals and were taught the census method.

2.2.4. Total count

We replicated Davidar's, 1976 method in order to compare the result with him. We divided the park into five sectors, identical to the sectors used in the foot surveys, each sectors was searched simultaneously by two groups of observers. Each group consisted of three observers. Survey effort time period on each sector was similar to the time spent by Davidar in each sector. The total count involved six observers who had served technicians on this study and had previous experience searching for tahr. We recorded all tahr sightings during the survey period. We eliminated sightings where we suspected a possibility of double counting.

3. RESULTS AND DISCUSSION

3.1. Hunters record

Totally 164 Nilgiri tahrs (*Hemitragus hylocrius*) hunted by game watchers and 4.9 average Nilgiri tahrs per year was hunted. Maximum 11 tahrs were hunted in the year 1907, 1931 followed by 10 Nilgiri tahr (*Hemitragus hylocrius*) shot in the year 1911, 1932. License holders can hunt one Sanddle back Nilgiri tahr (*Hemitragus hylocrius*).

3.2. Census counts

The census was carried out with forest Department and using the outer bound method (Robson and Whitlock, 1964). We estimated that the park and its surrounding areas contains between 374 at first trip and 553 in second trip.

3.3. Total counts

Total counts were conducted from 2007 to 2008. Myself and other researcher spent a total of 584.7 observer-hour spent and sighted 301 tahrs in 2007. During the year 2008, we spent of 618.93 and sighted off 337 tahrs. The maximum tahr was observed in Western catchment sector in 2007 sighted of 85 individuals and in 2008 hightest observed in Nadugani sector of 92 Nilgiri tahr (*Hemitragus hylocrius*).

3.4. Foot survey

Based on the sector wise percentage of 24.1% in the sector I, 23.3% in the sector II, 27.5% in the sector III, 23% in the sector IV and 2.3% in the sector V. Every hundred females 14% Saddleback, 12.66% Dark Brown Male, 22.66% Light Brown Male in 2007 was recorded. Based on the sector wise percentage of 21.8% in the sector I, 17.2% in the sector II, 26.7% in the sector III, 31% in the sector IV, 3% in the sector V. Every hundred females 12.57% Saddleback, 9.43% Dark Brown Male, 20.75% Light Brown Male in 2008.

3.5. Kids record

Totally 357 Nilgiri tahr sighted in the year 2007 and group sighted 36 tahrs at Mukurthi and least sighted in the Madipumalai only 3 Nilgiri tahr (*Hemitragus hylocrius*). Maximum kids recorded in

Chinna Mukurthi 10 kids in a group and maximum 22 kids seen in the month of November followed by December 16 kids in the year 2007 and minimum 2 kids seen in the month of October. Maximum kids recorded in Karaiguhai 9 kids in a group and maximum 31 kids seen in the month of November followed by February 24 kids in the year 2008.

4. CONCLUSION

In the present study, we observed the foot surveys conducted in 2007 and 2008 we estimated the population size as 358, 422 individuals respectively. We spent 2010 hours in 2007, 1980 hours in 2008 conducting theses surveys and to obtain detailed group composition counts. Because we repeatedly obtained the same sex and age composition of each groups in the various areas, we feel that we had seen 80% of animals. Based on our results compared with Davidar's 1976 estimated of 450 Nilgiri tahr. In addition overall population of Nilgiri tahr within the park is decline due to biotic, abiotic and manmade activities. Grouping pattern of Nilgiri tahr primarily based on seasonal influence and individual fitness. We found that more Nilgiri tahr in the north sector of the park but historically the south sector contains high density. The age specific mortality high in yearling of Nilgiri tahr in Mukurthi National park. The present study suggested that predator accounts for almost all mortality experienced by Nilgiri tahr. We found that abiotic factors influence the birth season of Nilgiri tahr. We found the food habits the Nilgiri tahrs are primarily grazers and grass constitute about 70% of their diet. Nilgiri tahrs are generally avoided of anthropogenic areas.

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Table 1. The population status of Nilgiri tahr (*Hemitragus hylocrius*) in the Mukurthi National Park for the period of 2007.

Location	N. tahr Population	6+ Years (SB)	5-6 Years (DBM)	2-4 Years (LBM)	2+ Years (AF)	1-2 Years (Y)	0-1 Years (K)	UI
Catchment trekking shed	24	1	1	4	13	1	4	-
Catchment No.2	27	2	1	4	12	4	3	1
Mukurthi Peak	14	1	-	1	6	3	2	1
Varagapallam West	13	1	1	1	6	1	3	0
Nilgiri Peak-base	33	2	1	5	11	6	6	2
Devabetta	14	-	-	1	5	2	6	-
Chinna Mukurthi	21	-	2	1	13	2	3	-
Catchment-view point	32	2	1	3	16	2	6	2
Karadiguhai	45	2	1	5	18	4	9	6
Madipumalai	10	2	-	-	-	-	-	8
Chettiparai	30	1	1	-	10	3	7	8
Nadugani East	53	2	1	2	10	3	12	23
Terace Estate*	27	1	-	-	-	-	-	26
Pandiar top*	18	1	1	2	9	2	3	-
Naduganimattam*	23	1	1	-	4	4	6	7
Pechakal Bettu*	38	-	1	2	16	12	7	-

*Represents Nilgiri Tahr (*Hemitragus hylocrius*) sighted outside the National park boundary
SB-Saddleback, DBM-Dark brown male, LBM-Light brown male, AF-Adult female, Y-Yearling, K-Kids, UI-Unclassified.

Table 2. The Population status of Nilgiri tahr (*Hemitragus hylocrius*) in the Mukurthi National Park for the period of 2008.

Location	N. tahr Population	6+ Years (SB)	5-6 Years (DBM)	2-4 Years (LBM)	2+ Years (AF)	1-2 Years (Y)	0-1 Years (K)	UI
Nilgiri Peak-base	26	1	2	4	12	2	4	1
Mukurthi Peak	23	2	1	5	11	3	1	-
Devabetta	12	1	1	2	6	1	-	1
Chinna Mukurthi	36	-	3	4	14	5	10	-
Pichal Bettu	5	-	-	1	2	-	2	-
Catchment View point	24	2	1	3	13	2	3	-
Catchment Trekking shed	23	1	1	3	9	3	4	2
Catchment No.2	24	2	1	2	10	2	6	1
Chettiparai	30	1	1	1	12	3	7	5
Madipumalai	3	2	1	-	-	-	-	-
Naduganimattam	16	1	1	-	4	4	6	-
Nadugani East	30	2	1	2	10	3	12	-
Karadiguhai	30	2	2	3	12	3	4	4
Terrace Estate*	20	2	1	-	8	-	4	5
Pandiar top*	18	1	1	2	9	2	3	-
Pecchakal Bettu*	31	-	1	2	16	12	-	-
Varagapallam West*	7	1	-	-	4	1	1	-

*Represents Nilgiri tahr (*Hemitragus hylocrius*) sighted outside the National park boundary
SB-Saddleback, DBM-Dark brown male, LBM-Light brown male, AF-Adult female, Y-Yearling, K-Kids, UI-Unclassified.

Table 3. Nilgiri tahr (*Hemitragus hylocrius*) Nilgiri tahr age and sex classification in Mukurthi National Park during 2007 to 2008.

Season	Animals encountered	Individuals classified	6+ years (SB)	5-6 years (DBM)	2-4 years (LBM)	2+ years (AF)	1-2 years (Y)	0-1 years (K)	Classified percentage
Winter	358	338	21	19	34	152	46	66	94%
Summer	422	393	21	15	32	176	63	86	93%

SB-Saddleback, DBM-Dark brown male, LBM-Light brown male, AF-Adult female, Y-Yearling, K-Kids, UI-Unclassified.

Table 4. Nilgiri tahr (*Hemitragus hylocrius*) Nilgiri tahr population structure towards 100 females in Mukurthi National Park during 2007 to 2008.

Season	**Animals encountered**	**Individuals classified**	**2+ years (AF)**	**6+ years (SB)**	**5-6 years (DBM)**	**2-4 years (LBM)**	**1-2 years (Y)**	**0-1 years (YO)**
Winter	358	338	100	13.8	12.5	22.37	30.26	43.42
Summer	422	393	100	11.9	8.52	18.18	35.8	48.86

SB-Saddleback, DBM-Dark brown male, LBM-Light brown male, AF-Adult female, Y-Yearling, K-Kids, UI-Unclassified.

Table 5. Showing sector wise population status Nilgiri tahr (*Hemitragus hylocrius*) in Mukurthi National Park during the period 2007-2008.

Sector	Sq.km	Animals encountered	Individuals classified	6+ years (SB)	5-6 years (DBM)	2-4 years (LBM)	2+ years (AF)	1-2 years (Y)	0-1 years (K)
I	4.5	76	70	5	5	8	35	3	9
II	5.3	95	95	2	5	12	43	11	8
III	11.95	101	92	6	4	9	44	6	14
IV	31.75	79	74	7	5	5	26	6	14
V	24.96	7	7	1			4	1	1
2007	78.46	358	338	21	19	34	152	46	66
I	4.5	92	88	5	3	8	35	10	17
II	5.3	73	73	1	3	4	33	16	6
III	11.95	113	107	6	4	11	54	7	13
IV	31.75	131	112	8	4	8	48	9	22
V	24.96	13	13	1	1	1	6	1	2
2008	78.46	422	393	21	15	32	176	63	86

SB-Saddleback, DBM-Dark brown male, LBM-Light brown male, AF-Adult female, Y-Yearling, K-Kids, UI-Unclassified.

Table 6. The total count records on Nilgiri tahr (*Hemitragus hylocrius*) at Mukurthi National Park during the period 2007 and 2008.

Sector	Areas	Number of N.tahr seen		Hours spent in Survey		N. tahr observers hours	
		2007	2008	2007	2008	2007	2008
I	Nilgiri Peak, Devabetta. Pandiar top	70	81	148.3	2.6	152.45	3.18
II	Mukurthi peak, Chinna Mukurthi, Pichal bettu, Peechakal bettu	67	63	83.2	4.23	118.15	2.59
III	WCIII, WCII, Chettiparai	85	88	152.05	3.84	122.25	7.08
IV	Madipumalai, Nadugani, Sispara	72	92	126.15	1.89	151.08	2.03
V	Varagapallam	7	13	75	0.15	75	0.55
	Total	301	337	584.7	12.71	618.93	15.43

Table 7. The census records Nilgiri tahr (*Hemitragus hylocrius*) on Mukurthi National Park during the 2007 census record.

Areas	1st Day	2nd Day	3rd Day
Nilgiri Peak	44	18	4
Devabetta	1	0	0
Terrace estate	0	0	0
Pandiar top	0	0	0
Mukurthi peak	18	0	0
Chinna Mukurthi	18	0	0
Pichal bettu	0	0	0
Pichakal bettu	0	37	0
Catchment view point	1	0	22
Catchment trekking shed	0	25	0

Catchment no 2	29	0	18
Chettiparai	5	7	0
Kudugadibetta & Kolaribetta	0	4	0
Madipumalai	1	0	0
Naduganimattam	7	2	0
Nadugani East	0	6	0
Karadiguhai	0	0	0
Total	124	99	44

Table 8. The census records Nilgiri tahr (*Hemitragus hylocrius*) on Mukurthi National Park during the 2008 census records.

Areas	1st Day	2nd Day	3rd Day	4th Day	5th Day
Nilgiri Peak	0	46	43	2	0
Mukurthi shoulder	0	1	40	0	0
Mukurthi Peak	0	0	8	10	0
Chinna Mukurthi	0	14	46	19	0
Devabetta	0	0	5	0	0
Ellamalai	0	0	3	0	0
Nadugani West	0	6	3	50	0
Nadugani East	0	0	13	53	0
Madipumalai	0	10	0	2	0
Sispara	0	0	0	3	0
Kingerhundi	0	0	2	0	0
Western Catchment no2	0	18	22	0	0
Western Catchment iii	0	19	16	11	13
Western Catchment ii	0	18	3	20	19
Chettiparai	0	24	8	0	0
Bangitapal	0	0	0	0	0
Total	0	156	212	170	32

Table 9. The three different methods showing the population status of Nilgiri tahr (*Hemitragus hylocrius*) in Mukurthi National Park during study period.

Method	2007	2008
Foot survey	377	426
Census method	374	553
Total count	301	337

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STUDY ON THE PLANT SPECIES, *ACORUS CALAMUS* FOR INSECTICIDAL PROPERTIES AGAINST THE FILARIAL VECTOR, *CULEX QUINQUEFASCIATUS* SAY. (DIPTERA: CULICIDAE).

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ABSTRACT

Leaf extract of the species, *Acorus calamus* was evaluated for the egg hatchability, larvicidal and pupicidal activity and the protein content of mosquito, *Culex quinquefasciatus* under the room temperature in the laboratory. Dosage value as expressed in ppm was 10 to 140 for *Culex quinquefasciatus*. A relationship was observed between the plant extract doses and percentage mortality. The percentage of egg hatchability decreased; larval and pupal mortality were found to be increased with increase in the dosage. Based on the probit analysis, the LC_{50} value of egg (99.15), I instar (42.24), IV instar (101.49) and pupae (121.57mg/mL) were recorded. The ovary protein content of the treated (0.083) was estimated to be very low when compared with that of the control (0.172mg/mL).

Keywords: *Culex quinquefasciatus, Acorus calamus,* LC_{50}, protein.

1. INTRODUCTION

Vector control is a serious concern in developing countries like India due to lack of general awareness, development of resistance and socio-economic reason. The role of mosquitoes is becoming increasingly important in the recent years because of change in ecology caused by human intervention.

Mosquitoes constitute a major public health menace as vectors of serious human diseases (Logankumar *et al.,* 2008). Of the various mosquito spread diseases, filariasis transmitted by *Culex quinquefasciatus* is dangerous and is spreading into wucherria bancrofti have taken epidemic form and have been reported from Tamil Nadu, West Bengal, Uttar Pradesh, Gujarat and Delhi (Kebra *et al.,* 1992). In recent years, scientists try a variety of botanical derivatives to eradicate many harmful insect pests including mosquitoes. Insecticidal activity of neem has been reported. Vector control is facing a threat due to the emergence of resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future (Nandita Chowdhury *et al.,* 2008).

Aedes are vectors for the pathogens of various diseases like dengue fever, dengue haemorrhagic fever and yellow fever (Rajmohan and Ramaswamy, 2007). Many authors world wide started large screening activity for using extracts of medicinal and herbaceous plants to control mosquitoes (Halawa, 2001; Das *et al.,* 2003; Choochote *et al.,* 2004). The plant species, *Acorus calamus* is a widely distributed neotropical shrub introduced to many parts of the tropics.

For the present study, this species was screened against egg hatchability, larval and pupal mortality of the mosquito *Culex quinquefasciatus.* So as to control the population of *Culex quinquefasciatus* by an eco-friendly approach.

2. MATERIALS AND METHODS

Fresh leaves of *Acorus calamus* were collected from the plants growing in agricultural lands. Leaves were washed, shade dried and ground in a mixture to form a fine powder. The 25g of the powder was then used for extraction in acetone in soxhlet apparatus. The extract was concentrated on water bath to evaporate the acetone. The filterate was considered as pure material and redissolved in acetone to form standard formulation. By further dilutions with required amount of water, different doses (ppm) were prepared.

Eggs of *Culex quinquefasciatus* were procured from the Research Laboratory of National Institute of Communicable Diseases (NICD), Mettupalayam, Coimbatore and brought to the laboratory and cultured. Eggs, first and fourth larval instars and pupae were harvested from the colony and were placed in different concentrations as biocide. Twenty individuals were used for each concentration. Eggs, larval instars and pupae were checked for mortality for every 24 hours. In the case of control only, carrier solvent was added. Food was provided in all the test beakers. Each test was

replicated for five times.The effect of leaf *Acorus calamus* on the egg hatchability, mortality of first and fourth larval instars and pupal mortality of *Culex quinquefasciatus* was studied. Following 24 hours were corrected for natural response by Abbott's formula (Abbott, 1925) as follows:

Busvin (1971) suggested that the critical doses of susceptibility can be estimated with sufficient accuracy from a probit / log concentration graph. Based on the log concentration and the probit mortality percentage values, regression equation was obtained. Using the regression, a straight line was fitted. Fitting of regression line and homogeneity of population were also tested employing chi-square (χ^2) test. By graphical interpolation, LC_{50} values of the leaf extract for 24 hours of exposure of egg, first and fourth instar larvae and pupae of *Culex quinquefasciatus* and their fiducial limits (95% upper fiducial limit and lower fiducial limit) were calculated.

Blood fed females survived through pupae treated with any concentration of plant extract was harvested at different hours. The ovaries were carefully dissected out and washed in physiological saline solution. The adhering water the tissues were removed using filter paper. The ovaries were weighed and homogenized in phosphate buffer (pH 7.0; 0.01M). The sample was then centrifuged at 5000rpm for 10 minutes and supernatant was taken for the estimation of protein by adopting standard methods for protein determination.

3. RESULTS AND DISCUSSION

Mortality values of egg, larvae and pupae treated with different concentrations (ranging from 10ppm to 140 ppm) of the leaf extract of *Acorus calamus* at the end of 24 hrs are given in Table 1-4 for egg, I instar, IV instar larvae and pupae of *Culex quinquefasciatus*. The LC_{50} values and their 95% upper and lower fiducial limits, and chi-square value of the leaf extract of *Acorus calamus* for 24h exposure of *Culex quinquefasciatus* are given in Table 5. Based on the probit analysis the 24 hr LC_{50} value of the leaf extract of *Acorus calamus* for egg, I instar and IV instar larvae and pupae of *Culex quinquefasciatus* was found to be 99.15, 42.24, 101.49 and 121.57 respectively (Fig.1). The ovary protein content of the treated was estimated to be very low when compared with that of the control (Table 6). An important criterion determine in the present study the enzyme activity declined invariably in all the treatments of *Culex quinquefasciatus* with *Acorus calamus* leaf extract. Decline in the ovary protein content will reduce the egg laying capacity of *Culex quinquefasciatus* where as inhibition of enzyme activity will help in the arresting of the developmental stages. Inhibitory effect of *Acorus calamus* leaf extract was found to be higher than that of the synthetic inhibitors. The visible morphological abnormalities occurred in treated were the larva were smaller than that of its control. Pupae survived through larval treatment frequently showed a variety of malformations like demelanized pupa with straight abdomen, partially melanised pupa with extended abdomen, dwarf pupa with retarded abdomaen, dechitinised pupa and inability of the adult to shed completely its exiuuvia which remained attached to its appendages. These results are in agreeing with the earlier findings made by many workers with botanicals for various properties (for oviposition avoidance, Tilak *et al*., 2005; larvicidal, Halawa, 2001; Khater, 2003; Saleh, 1995, adulticidal, Choochote *et al*., 2004 and repellent activities, Choochote *et al.,* 2004; Prakash *et al*., 2000). As the botanical insecticides for including the extract of *C. odarata* are biodegradable and harmless to the environment, pest – specific and relatively harmless to non-target organisms (Su and Mulla 1998; Sivagnaname and Kalyana Sundaram, 2004; Sun *et al*., 2006) they are more eco-friendly. The three active principle compounds reported in the study species *Acorus calamus* such as Augustineolide, 3 – β – 6 hydroxy, dihydroxy cartin and 6 – acetoxihumininolide are determined to have mosquitosidal properties which is perhaps be a reason for the medicinal use of this species in terms of mosquito repellent (Logankumar, 2006). The results of the present study, indicate that the leaf extract of the species, *Acorus calamus* caused low percentage of egg hatchability and high percentage of larval and pupal mortality. Hence the large biomass of *Acorus calamus* available in Southern India can be used in the pharmacological industries to obtain effective repellent to control mosquito population is an ecofriendly manner.

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Table 1. Effect of partially purified sample of *Acorus calamus* against the egg hatchability of *Culex quinquefasciatus.*

No of eggs exposed	Concentration (ppm)									
	70		80		90		100		110	
	h	uh	h	uh	h	uh	h	uh	h	uh
20	17	3	14	6	11	9	6	14	3	17
20	16	4	12	8	10	10	7	13	2	18
20	17	3	11	9	10	10	5	15	3	17
20	18	2	13	7	11	9	4	16	1	19
20	15	5	11	9	10	10	5	15	4	16
Mean	16.6	3.4	12.2	7.8	10.4	9.6	5.4	14.6	2.6	17.4
SD	1.14	1.14	1.30	1.30	0.55	0.55	1.14	1.14	1.14	1.14
Mean %	83	17	61	39	52	48	27	73	13	87

h - hatched, un - unhatched

Table 2. Effect of partially purified sample of *Acorus calamus* against the I Instar larvae of *Culex quinquefasciatus.*

No of larvae exposed	Concentration (ppm)									
	10		20		30		40		50	
	Alive	dead	Alive	dead	Alive	dead	Alive	dead	Alive	dead
20	19	1	17	3	12	8	11	9	2	18
20	18	2	15	5	11	9	10	10	4	16
20	19	1	16	4	12	8	10	10	3	17
20	16	4	17	3	13	7	12	8	4	16
20	17	3	15	5	11	9	10	10	5	15
Mean	17.8	2.2	16	4	11.8	8.2	10.6	9.4	6.4	13.6
SD	1.30	1.30	1.00	1.00	0.84	0.84	0.89	0.89	1.14	1.14
Mean %	89	11	80	20	59	41	53	47	32	68

Table 3. Effect of partially purified sample of *Acorus calamus* against the IV instar larvae of *Culex quinquefasciatus.*

No of larvae exposed	Concentration (ppm)									
	80		90		100		110		120	
	Alive	dead	Alive	dead	Alive	dead	Alive	dead	Alive	dead
20	19	1	15	5	11	9	8	12	3	17
20	18	2	12	8	10	10	9	11	2	18
20	16	4	11	9	10	10	8	12	1	19
20	19	1	13	7	11	9	7	13	2	18
20	17	3	12	8	12	8	6	14	4	16
Mean	17.8	2.2	12.6	7.4	10.8	9.2	7.6	2.4	2.4	17.6
SD	1.30	1.30	1.52	1.52	0.84	0.84	1.14	1.14	1.14	1.14
Mean %	89	11	63	37	54	46	38	62	12	88

Table 4. Effect of partially purified sample of *Acorus calamus* against the pupae of *Culex quinquefasciatus.*

No of larvae exposed	Concentration (ppm)									
	100		110		120		130		140	
	Alive	dead	Alive	dead	Alive	dead	Alive	dead	Alive	dead
20	18	2	15	5	12	8	9	11	5	15
20	19	1	14	3	10	10	8	12	3	17
20	18	2	11	9	10	10	9	11	2	18
20	17	3	12	8	10	10	6	14	3	17
20	16	4	11	9	11	9	7	13	4	16
Mean	17.6	2.4	12.6	6.8	10.6	9.4	7.8	12.2	3.4	16.6
SD	1.14	1.14	1.82	2.68	0.89	0.89	1.30	1.30	1.14	1.14
Mean %	88	12	63	34	53	47	39	61	17	83

Table 5. 24 hours LC_{50} values (ppm) and their 95 % Fiducial (upper and lower) regression equation and Chi-square (χ^2) values of the partially purified extract of *Acorus calamus* for the different developmental stages of *Culex quinquefasciatus.*

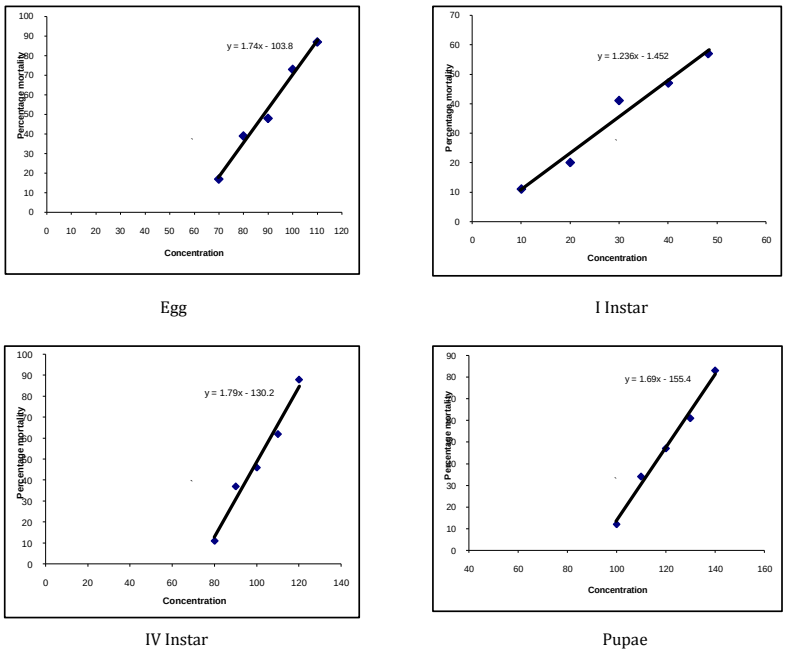
Stages	LC_{50} (ppm)	95% Fiducial limit (ppm)		χ^2	$\overline{X}$	SE
		Upper	Lower			
Egg	99.15	103.74	95.65	2.57	52.8	3.88
I instar	42.24	45.58	39.75	6.66	30.2	11.52
IV instar	101.49	105.38	107.61	4.58	48.8	5.25
Pupa	121.57	125.43	117.14	4.25	47.4	3.11

Table 6. Determination of ovary protean during different hours of blood meal on control and treatment with *Acorus calamus* leaf extract in *Culex quinquefasciatus.*

Different hours after blood meal (mg/mL)											
5		10		15		20		25		30	
C	T	C	T	C	T	C	T	C	T	C	T
0.154	0.083	0.188	0.121	0.197	0.131	0.200	0.142	0.241	0.151	0.168	0.086
0.169	0.098	0.194	0.119	0.199	0.148	0.211	0.144	0.258	0.155	0.179	0.084
0.154	0.105	0.187	0.100	0.198	0.127	0.196	0.138	0.221	0.159	0.168	0.084
0.172	0.110	0.161	0.115	0.180	0.135	0.203	0.156	0.260	0.167	0.174	0.077
0.162	0.097	0.183	0.117	0.193	0.135	0.209	0.146	0.268	0.158	0.172	0.86
Mean :											
0.162	0.099	0.183	0.144	0.193	0.135	0.204	0.145	0.249	0.158	0.172	0.083
SD± :	0.009	0.012	0.008	0.008	0.008	0.006	0.007	0.019	0.006	0.005	0.003
0.008											

C – Control; T - Treatment

Fig 1. Regression line of log concentration of *Acorus calamus* partially putified extract vs. percent egg hatchability, mortality of larvae and puape of *Culex quinquefasciatus.*



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ANTIOXIDANT POTENTIAL DETERMINATION OF *PIPER BETLE* AND *CISSUS QUADRANGULARIS*

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ABSTRACT

India has vital deposit of various herbs and is used in many parts of the country as traditional medicinal treatment like Ayurveda, Unani, Siddha and Homeopathy. With increasing problems of side effects related to chemical drug usages and increasing activity of over drug usages has led to multidrug resistant organisms. In this research we focused on using the plant sources like *Piper betle* leaves and *Cissus quadrangularis* stems for understanding their antioxidant scavenging activity. We have extracted the vital compounds using cold percolation method and the methanolic extract was chosen for various antioxidant activity like DPPH, $ABTS^{+}$, FRAP, Hydrogen peroxide and hydroxyl radical scavenging activity. We have found that both the plant extracts showed higher activity and IC_{50} value for both the extracts in all the tests showed nearly 60 µg mL^{-1} and the graph was plotted using the percentage of inhibition scavenging activity vs the concentration taken for each plant extracts.

Keywords: *Piper betle, Cissus quadrangularis,* $DPPH^{\bullet}$, $ABTS^{\bullet+}$, FRAP, Hydrogen peroxide and hydroxyl radical.

1. INTRODUCTION

India is a country with vital deposit of various herbs and people utilize these herbs in their food recipes as spices or as vegetable leaves from time immemorial. The World Health Organization (WHO) estimates that plant or their extracts are used in the traditional folk medicine and used 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 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 betle* and *Cissus quadrangularis* as the source with medicinal property for our research. 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. This causes various trouble related to gastric problems as well as imbalance due to free radicals. We have aimed to understand the scavenging potential of these plants as they are commonly used in India as a source for treating gastric diseases and acidity problems in various parts of South India as well as in tribal areas.

2. MATERIALS AND METHODS

2.1. Collection of herbal plants

Piper betle leaves and *Cissus quandrangularis* stems were collected from Tirupur flower market and subjected for plant authentication at Botanical Survey of India, Coimbatore, Tamil Nadu.

2.2. Processing of Plant leaves and stem 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 avoid the medicinal property of any medicinal plant. The plant leaves and stems were stored in dark for a period of 3 weeks and were powdered. The powders of plant leaves and stem was sieved and stored in dark bottle. About 3 g of powdered plant leaves and stem were separately weighed and added to 30 ml of different solvents in increasing order of polarity such as petroleum ether, chloroform, ethanol, hexane, methanol and distilled water. They were kept in shaking at 200 rpm for 3 days and the extraction was done using muslin cloth. The filtrate was evaporated in dark and scrapped

and stored in dark bottle. It was subjected to antioxidant scavenging activity and subjected for future research against pathogens.

2.3. Evaluating the various antioxidant properties of extracted P. betle and C. quadrangularis by in vitro free radical scavenging activity

2.3.1. DPPH• scavenging activity

The 2,2-diphenyl-1picryl hydrazyl (DPPH) scavenging activity of *P. betle* and *C. quandrangularis* is performed using the method described by Blois, in 1995. Various concentrations of samples were taken and as standard Vitamin C were used in different test tubes. The volume was adjusted to 500 µL by adding methanol. About 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. Changes in the absorbance of the samples were measured at 517 nm. The percentage of inhibition radical scavenging activity was measured by the formula:

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

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). 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

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

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. H_2O_2 concentration was determined using spectrophotometer from its absorption at 230 nm. 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:

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

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). 3 ml of Nash reagent was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectrophotometrically at 412 nm against reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by the following formula.

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

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 ferrous sulphate in the range 0.1mM ethanol solutions. The concentrations of $FeSO_4$ were in turn

plotted against concentration of standard antioxidants.

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

3. RESULTS AND DISCUSSION

The collected *P. betle* leaves and *C. quadrangularis* stem were taken to Botanical Survey of India, Coimbatore and authentication no. for the plant is BSI/SRC/5/23/2013-14/Tech./1893. The processing and extraction of the plants leaves and stems were done accordingly and the methanolic extract of both the plants were subjected for further scavenging activity analysis based on the work done by Gupta and Ray (2004).

3.1. Evaluating the various antioxidant properties of extracted Piper betle and Cissus quadrangularis by In vitro free radical scavenging activity

Methanolic extracts of *P. betle* and *C. quadrangularis* 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, $ABTS^{+}$, 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 of *C. quadrangularis* was higher than *P. betle.* The results were observed to have higher percentage of inhibition for the extracts and the IC_{50} value was observed as 60µg/ml in all the tests (Fig. 1, 2, 3, 4, and 5).

This shows that *P. betle* and *C. quadrangularis* has higher scavenging activity as compared with the results of Gupta and Ray (2004) and Kumar *et al.* (2010). Based on the experimental outcome of Abrahim *et al.* (1993), the antioxidant activities of the leaves of *P. betle* showed inhibitory effect on the proliferation of the breast cancer cell line, MCF-7. 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).

4. CONCLUSION

This proves that both the plants *P. betle* and *C. quadrangularis* have higher scavenging activity using the methanolic extract of plant leaves and stems. 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.

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Table 1. DPPH$^{\bullet}$ radical scavenging activity.

Concentration µg/mL	% of Inhibition of Standard (Vitamin C)	% of Inhibition of *Piper betle*	% of Inhibition of *Cissus quadrangularis*
20	8.882	8.998	10.010
40	27.844	11.425	23.761
60	40.119	35.288	48.230
80	58.882	47.118	75.935
100	85.828	75. 530	79.777

Table 2. ABTS$^{\bullet+}$ radical scavenging activity

Concentration µg/mL	% of Inhibition of Standard (Vitamin C)	% of Inhibition of *Piper betle*	% of Inhibition of *Cissus quadrangularis*
20	7.280	12.899	7.371
40	35.760	29.852	25.921
60	53.961	50.859	53.961
80	76.552	73.710	76.552
100	89.186	76.289	89.186

Table 3. Hydroxyl radical scavenging activity

Concentration µg/mL	% of Inhibition of Standard (Vitamin C)	% of Inhibition of *Piper betle*	% of Inhibition of *Cissus quadrangularis*
20	6.216	1.722	8.151
40	23.280	15.843	18.254
60	57.407	29.735	34.902
80	79.761	62.571	53.960
100	89.682	75.545	69.345

Table 4. Hydrogen peroxide scavenging activity

Concentration µg/mL	% of Inhibition of Standard (Vitamin C)	% of Inhibition of *Piper betle*	% of Inhibition of *Cissus quadrangularis*
20	16.939	18.961	9.315
40	31.420	45.012	27.947
60	45.264	58.779	48.062
80	58.105	66.611	58.944
100	78.688	71.393	73.371

Table 5. Ferric Reducing Antioxidant Power Assay (FRAP)

Concentration µg/mL	OD value at 595nm of Standard (Vitamin C)	OD value at 595nm of *Piper betle*	OD value at 595nm of *Cissus quadrangularis*
20	0.18	0.26	0.198
40	0.362	0.394	0.322
60	0.62	0.467	0.576
80	0.713	0.519	0.622
100	0.96	0.73	0.79

Fig. 1. DPPH radical scavenging activity of *P. betle* and *C. quadrangularis*

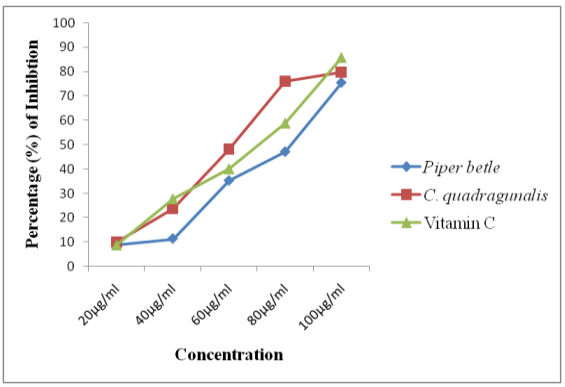


Fig. 2. ABTS⁺ radical scavenging activity of *P. betle* and *C. quadrangularis*

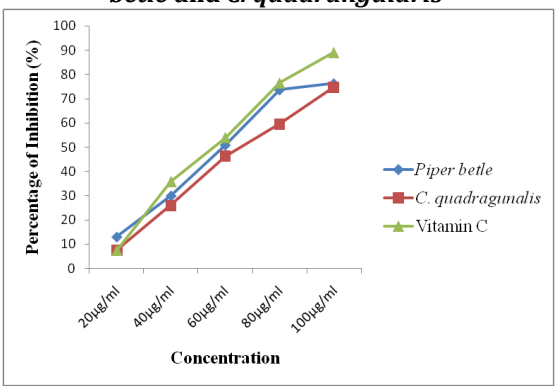


Fig. 3. Hydroxyl radical scavenging activity of *P. betle* and *C. quadrangularis*

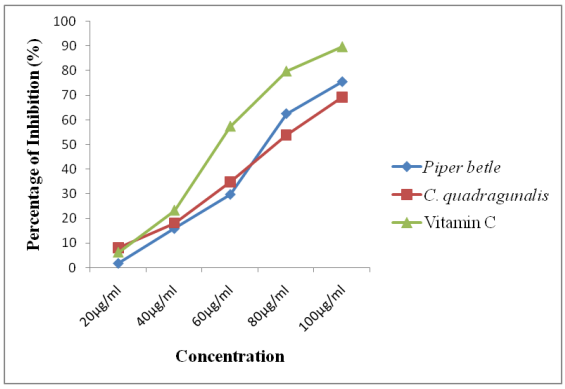


Fig. 4. Hydrogen Peroxide radical scavenging activity of *P. betle* and *C. quadrangularis*

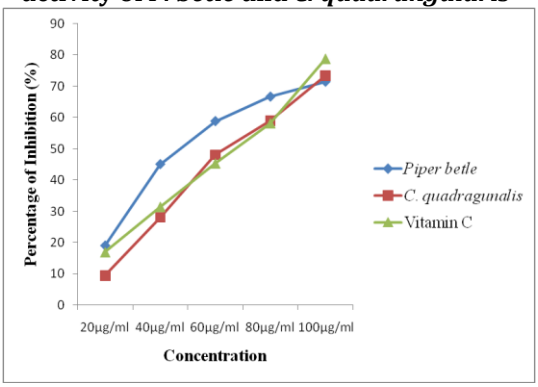
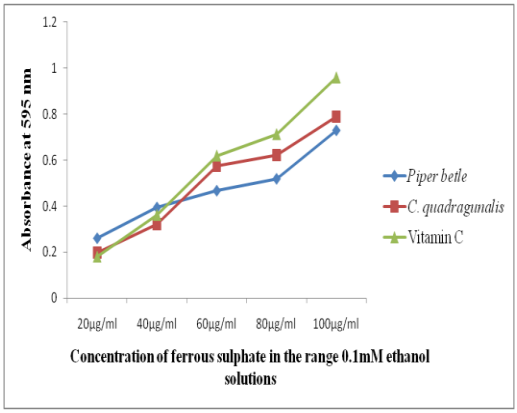


Fig. 5. FRAP radical scavenging activity of *P. betle* and *C. Quadrangularis*



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EFFECT OF *ANANUS COMOSUS* LINN. PEEL EXTRACT ON 7, 12 DIMETHYL BENZ(A)ANTHRACENE (DMBA) INDUCED MAMMARY CARCINOMA IN FEMALE SPRAGUE DAWLEY RATS

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ABSTRACT

Breast cancer is one of the leading second most prevalent malignant tumors in the world among women and its incidence continue to increase every year. Oxidative stress plays a key role in many cancers including breast cancer, oral cancer etc. Hence the use of natural antioxidants in day to day life is very important privilege to quenching those diseases caused by free radicals. Therefore the present study was undertaken to evaluate the effect of *Ananus comosus* linn. peel extract on hepatic and renal expression of oxidative stress enzymes in 7, 12 dimethyl benz(α)anthracene induced mammary carcinoma. Female Sprague Dawley rats weighing 180 ± 10g were used for experimental purpose. The animals were divided in to five groups, group I served as control, group II animals were treated 7, 12-dimethyl-benz[*a*]anthracene, group III and group IV animals were treated with ethanolic extract of *Ananus comosus* (250 mg/kg body weight) and standard drug tamoxifen (10 mg/kg body weight) for 30 days after 90 days of tumor induction, group V animals were treated with ethanolic extract of *Ananus comosus* alone (250 mg/kg body weight) for 30 days. After the experimental period, the animals were sacrificed by cervical decapitation. Hepatic and renal tissues were used to analyze for protein, enzymic and non enzymic antioxidants levels. Rats treated with plant extract showed significant increased in antioxidant levels when compared to cancer bearing untreated rats. In conclusion, it can be inferred that ethanolic extract of *Ananus comosus* peel positively modulated the antioxidant activity by quenching and detoxifying the free radicals induced by 7, 12-dimethylbenz(α)anthracene in both liver and kidney tissues.

Keywords: Breast cancer, *Ananus comosus*, 7, 12-dimethyl-benz[*a*]anthracene, enzymic and non enzymic antioxidants.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are products formed by incomplete combustion of organic matter. Sources of PAHs include industrial and domestic oil furnaces, gasoline, char-broiled meat, tobacco smoke, diesel exhaust. They are widely distributed in our environment and are implicated in various types of cancer (Chan *et al.,* 2002). Enzymatic activation of PAHs leads to the generation of active oxygen species like peroxides and superoxide anion radicals, which induce oxidative stress in the form of lipid peroxidation. Previous studies have shown that these PAHs can cause genotoxic and capable of forming carcinogen-DNA adducts in both human and animal tissues. Once these chemicals are consumed, our body will metabolize and transform these compounds into DNA-attacking mutagens. Higher amounts of PAH-DNA adducts have been found in human breast tumors than in normal breast tissues (Nandakumar *et al.,* 2011). The PAH 7, 12-dimethyl-benz[*a*]anthracene (DMBA) acts as a potent carcinogen by generating various reactive metabolic intermediates leading to oxidative stress (Bishayee *et al.,* 2000).

Oxidative stress plays a key role in many cancers including breast cancer, oral cancer etc. Several experimental investigations as well as clinical and epidemiological findings have proven that accumulation of this oxidative stress related proteins such as singlet oxygen ($^1O_2$), superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) to our body and their improper removal of those proteins is the hallmark of cancer development. Reactive oxygen species are produced by several ways like during infection, inflammatory conditions and exposure to toxins, pollutants and radiations (Ishii, 2007; Laviano *et al.,* 2007). Certain aldehyde such as malondialdehyde

(MDA) is the end product of lipid peroxidation (LPO) arising from the free radical generation leading to the degradation of polyunsaturated fatty acids can cause cross-linking in lipids, proteins and nucleic acids subsequently faster the growth of tumor cells (Ozben, 2007; Valko *et al.,* 2006). Excess free radicals can cause tissue damage and hypoxia as a result of not only overexposure to environmental factors but also from a lack of antioxidants or destruction of free radical scavengers. Hence the use of natural antioxidants in day to day life is very important privilege to quenching those diseases caused by free radicals (Scheibmeir *et al.,* 2005).

Numerous plants tend to have antioxidant potential property among that *Ananas comosus* (L.) is belonging to the family Bromeliaceae and it is broadly cultivated in the tropical areas of the world. Pineapple has also been known for a number of beneficial biological activities like antioxidative, anticancer, anti-browning, anti-inflammatory and anti-platelet activities. The enzyme complex of *Ananus comosus* called bromelain is known for its clinical applications particularly modulation of tumor growth, blood coagulation and anti-inflammatory effect (Chaisakdanugull *et al.,* 2007). Pineapple has been extensively used in foods or for health benefits.

Looking towards the medicinal properties of this plant, the present study is undertaken to obtain insight into the possible anti-cancer activity of *Ananas comosus* against DMBA-induced mammary carcinogenesis which related to oxidative stress parameters in rats.

2. MATERIALS AND METHODS

2.1. Collection of Plant material

Fresh pineapple plant was collected from Coimbatore, Tamil Nadu, India. The plant was authenticated by Dr. P. Sathyanarayanan, Botanical survey of India, TNAU Campus, Coimbatore and the voucher specimen No.BSI/SRC/5/23/2011/Tech-515. Fresh peel part of the sample was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles.

2.2. Sample Extraction

100g of dried plant powder (from 1kg of *A.comosus* peel) was extracted in 500ml of ethanol in a water shaker for 72hrs. Repeatedly extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated to dryness by using a rotary vacuum evaporator at 40-50^{o}C and stored at 0-4^{o}C in an air tight container.

2.3. Chemicals

7, 12 Dimethyl benz(α)anthracene, oxidized glutathione and reduced glutathione were purchased from Sigma Chemical Company, USA. All other chemicals used were of analytical grade.

2.4. Experimental Animals

Female Sprague Dawley rats weighing 180 ± 10g were purchased from Karpagam University, Coimbatore and housed in plastic cages. The animals were maintained under controlled environmental condition on alternative 12h dark/light cycle. Commercial pelleted feed and water ad libitum were given to animals. All the experiments were carried out according to the guidelines recommended by the committee for the purpose of control and supervision of Experiments on Animals (CPCSEA) and approved by IAEC, Government of India for the use of Sprague dawley rats as an animal model for cancer activity.

2.5. Experimental design

The animals were divided in to five groups of 6 animals each. Group I animals served as control, Group II animals were treated with 25 mg of DMBA in 1.0 ml olive oil by gastric incubation, to induce mammary cancer. After 90 days of tumor induction Group III and Group IV animals were treated with ethanolic extract of *Ananus comosus* (250 mg/kg body weight) and standard drug tamoxifen (10 mg/kg body weight) for 30 days. Group V animals were treated with ethanolic extract of *Ananus comosus* alone (250 mg/kg body weight) for 30 days.

2.6. Collection of hepatic and renal tissue

After the experimental period, the animals were sacrificed by cervical decapitation. Liver and kidney tissues were immediately excised. A 10% homogenate was prepared in 0.1 M Tris-HCl buffer pH 7.4 using Potter Elvehjem homogenizer with Teflon pestle.

2.7. Biochemical analysis

Liver and kidney homogenates were used to analysis the various biochemical parameters like protein (10% homogenate was prepared in 0.1 M Tris-HCl cell lysis buffer pH 7.4, from that 0.1ml of sample was taken for the analysis of protein) content was measured according to the method of Lowry *et al.,* (1951). Superoxide dismutase was measured as Das *et al.,* (2000). Catalase was measured by the method of Sinha, (1972). Glutathione peroxidase was measured by Rotruck *et al.,* (1973). Glutathione reductase was measured by Beutler, (1984). Glutathione-S-transferase was measured by

Mannervik, (1985). Glucose 6-phosphate dehydrogenase was measured by Balinksy and Bernstein, (1963). Total reduced glutathione was measured by Moron *et al.,* (1979). Ascorbic acid was measured by Omaye *et al.,* (1979). Vitamin E was measured by Rosenberg, (1992) and Lipid peroxidation was measured according to the method given by Buge and Aust, (1978).

2.8. Statistical analysis

The results are expressed as mean ± standard deviation (S.D). Difference between the groups was assessed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test using the SPSS 10.0 version software package for windows. The values were considered statistically significant if p value was less than 0.05 ($p<0.05$).

3. RESULTS AND DISCUSSION

The synthetic polycyclic aromatic hydrocarbon 7, 12 dimethylbenz(∞)anthracene (DMBA) is a powerful carcinogen which is selectively active in sites such as mammary glands, skin, kidney and liver, and has been widely used as a prototype carcinogen in experimental animal models. After exposure to DMBA, stable DNA-carcinogen adducts may be found in various tissues including the kidney, liver and mammary gland (Song *et al.,* 2000). Oxidative stress, an imbalance in oxidant and antioxidant status, has been well documented in several cancers including breast cancer. DMBA on metabolic activation excessively generates reactive oxygen species causing severe damage to DNA contributing to carcinogenesis (Kolanjiappan and Manoharan, 2005).

Chemoprevention, a novel approach in recent cancer research, deals with the prevention, inhibition, suppression and reversal of carcinogenic process by using natural plant products or their constituents and synthetic chemical agents (Senthil *et al.,* 2007). In recent years, research scientists have focused their attention on cancer chemoprevention using medicinal plants, due to the fact that the natural products are non toxic, having less side effects and affordable at low cost. Medicinal plants and their bioactive constituents exert their chemopreventive efficacy by preventing metabolic activation of carcinogens, increasing detoxification of the carcinogen, blocking the interaction of ultimate carcinogen with cellular macromolecules and by suppressing the clonal expression of the neoplastic cells and those posses intrinsic antioxidants, radical trapping and anti-inflammatory properties, can act as potent chemopreventive agents (Leena and Jaindra, 2003).

The effect of *Ananus comosus* peel extract on protein level in control and experimental group of animals were represented in table 1. The levels of protein in liver and kidney tissue of breast cancer induced group (43.64 ± 0.72^{a}, 27.12 ± 3.34^{a}) showed significantly depleted ($p<0.05$) when compared to control animals (71.77 ± 2.68^{c}, 52.64 ± 3.35^{c}). On the other hand, oral administration with ethanolic extract of *Ananus comosus* peel (63.27 ± 2.18^{b}, 45.19 ± 2.40^{b}) and tamoxifen ($66.83\pm1.10b^{c}$, 51.04 ± 4.05^{bc}) (standard drug) treated groups showed significant increased protein level when compared with group II rats; while there is no significant changes ($p<0.05$) were observed in plant extract treated alone group (group V, 73.1 ± 5.38^{c}, 54.76 ± 2.40^{c}) animals when compared to control animals.

Proteins are an important phenomenon in normal as well as in cancer conditions. The highest rate of synthesis of tissue proteins and major protein mass of the organism is severely affected in cancer cachexia. Cancer cachexia results in progressive loss of body weight, which is mainly accounted by wasting of host body compartments such as skeletal muscle and adipose tissue (Argiles and Aczon-Bieto, 1988; Khan and Tisdale, 1999). In the present investigation, decreased protein levels were observed and this may be due to the utilization of host proteins for the progression of tumor in DMBA induced mammary cancer bearing animals. This is well documented with the previous reports of Nandakumar *et al.,* (2011). Obstinately, treatment with *Ananus comosus* peel extract significantly prevents the protein degradation rate and thus increases the total protein content by modulating protein synthesis which inevitably proves that plant extract has some key modulatory effect by intervening in the synthesis of proteins and maintains its level in a significant amount.

Table 2 and 3 indicate the concentration of Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) in the hepatic and renal tissues of control and experimental groups. Decreased in the antioxidant status like SOD, CAT and GPx were noticed in cancer induced animals (Group II) as compared to control (Group I). However, oral administration of *Ananus comosus* and tamoxifen treated group (Group III and IV) reverted the concentration of those levels to near normal range. Animals treated with plant extract alone (Group V) showed no significant difference in the levels of SOD, CAT and GPx, as compared to control animals.

SOD acts as the first line of defense against superoxide radicals, which dismutates two superoxide radicals to H_2O_2 and O_2. In addition, CAT and GPx act as supporting antioxidant enzymes by converting H_2O_2 to H_2O, thereby providing protection against ROS. GPx is another endogenous antioxidant selenoprotein present in the cytosol and mitochondrial matrix that participates in the defense mechanism (Vasquez-Garzon *et al.,* 2009). The reduction in activity of these enzymes may be caused by the increase in radical production during DMBA metabolism. In the present investigation, an increase in MDA formation was presumably associated with increased ROS, consistent with the observation that these free radicals reduce the activity of hepatic and renal SOD. In corroborate with the previous studies of Singha *et al*., (2009), biochemical results of SOD, CAT and GPx showed a decrease in activity in cancer bearing rats compared to control rats which may be due to the over production of radicals during mammary cancer.

A significant fall in Glutathione reductase (GR), Glutathione-S transferase (GST) and glucose-6 phosphate dehydrogenase (G6PD) activities were noticed in liver and kidney tissues in the group-II animals as compared to normal group. Treatment with *Ananus comosus* and drug resulted in an enhanced levels of GR, GST and G6PD ($p<0.05$) in such groups when compared to cancer bearing animals. Upon treatment with *Ananus comosus*, there is no significant changes of these non enzymatic antioxidants in the plant extract alone-treated group (Table 4 and 5).

GST catalyzes the conjugation of xenobiotic electrophilic substances with GSH to form the corresponding GSH-S-conjugate (Yeh *et al.,* 2005). Glucose-6-phosphate dehydrogenase (G6PD) is responsible for generating the NADPH, required for the recycling reaction of GSSG to GSH (Felix *et al.,* 2003). In the present investigation DMBA intoxicated rats showed a significant fall in the activity of these antioxidant enzymes in liver and kidney tissue might be due to the over production of ROS. Pretreatment with ethanolic extract in cancer bearing animals intoxicated rats show a significant recovery of hepatic enzymatic antioxidant systems (Mathivadhani *et al.,* 2007).

Changes in antioxidants in hepatic and renal tissues of rats induced with DMBA showed significant ($p<0.05$) depletion in total reduced glutathione, vitamin E and vitamin c (Table 6 and 7). Interestingly, the treatment with *Ananus comosus* extract and standard drug has restored normal levels of non enzymatic antioxidants when compared with group II animals. Treatment with plant extract alone exhibited no significant changes on the enzyme activities compared to normal control group.

GSH plays a critical role in maintaining cell viability through regulation of inner membrane permeability by maintaining sulfhydryl groups in the reduced state. The observed lower level of glutathione in mammary carcinoma bearing rats may result in enhanced lipid peroxidation that can cause increased GSH consumption during an early consequence of oxidative stress in cancer rats, which is in line with earlier investigation (Dean *et al.,* 1997). In the present observation, vitamin C and vitamin E were found to be significantly decreased in DMBA induced animals and reverted to near normal levels by administration of *Ananus comosus* peel. The decreased vitamin C and vitamin E in cancer bearing rats might be due to increased free radical generation and tocopheroxyl radicals during their intervention with ROS. Absence of sufficient regenerating systems such as glutathione and NADH leads to decreased vitamin C and vitamin E levels are in agreement with the report of Arulkumaran *et al.,* (2007).

Table 8 represents the toxic effect of DMBA was justified by the significant ($p<0.05$) enhanced in the lipid peroxidation (LPO) levels when compared to negative control group. The antioxidant effect of plant extract and standard drug was observed by significant ($p<0.05$) deprived in the activity of LPO when compared to DMBA positive control. No changes were found in extract alone treated group (group V) when compared with group I control group.

Lipid peroxidation (LPO) refers to the reaction of oxidative deterioration mainly it affects the polyunsaturated fattyacids (Seckin et al., 1993). LPO is the presumptive marker for free radicals generation and the development of oxidative damage. It has been measured by indirectly measuring the thiobarbituric acid (TBA) reactive material, MDA and it is one of the end products of LPO. The increased levels of LPO in group II cancer-bearing animals of the present investigation may be due to free radicals produced by DMBA administration and is consistent with the previous reports of Devis and Kuttan, (2001); Bhuvaneswari *et al.,* (2004); Premalatha *et al.*, (1997) are of the opinion that enhanced LPO and decreased enzymic antioxidant levels were observed in cancer condition. This may be due to free radicals induced by DMBA. High levels of free radicals (ROS) are involved to damage many biomolecules and exert diverse cellular and molecular effects including

mutagenicity, cytotoxicity and changes in gene expression that led to both in the initiation and promotion stage of carcinogenesis also their biochemical reactions are involved in each stage of the metabolic process and are associated with cancer development (Behrend *et al.,* 2003). From the present study, it is evidenced that increased level of LPO was found in cancer bearing animals when compared to control group which is consistent with the previous findings of Anbuselvam *et al.,* (2007).

4. CONCLUSION

In conclusion, it can be inferred that ethanolic extract of *Ananus comosus* peel positively modulated the antioxidant activity by quenching and detoxifying the free radicals induced by 7, 12-dimethylbenz(α)anthracene. Considering the antioxidant property of this extract, the bioactive compounds derived from this *Ananus comosus* peel can be supplemented with anticancer medicines in future. Further investigations on these extract on anticancer mechanisms are currently in progress.

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Table 1: Effect of *Ananus comosus* on protein level in liver and kidney of control and experimental animals

Groups	Liver	Kidney
Control	71.77±2.68^{c}	52.64±3.35^{c}
DMBA induced	43.64±0.72^{a}	27.12±3.34^{a}
DMBA+*A.comosus*	63.27±2.18^{b}	45.19±2.40^{b}
DMBA+Tamoxifen	66.83±1.10b^{c}	51.04±4.05^{bc}
A.comosus alone	73.1±5.38^{c}	54.76±2.40^{c}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at p< 0.05 (DMRT)
Comparison between the groups
a- comparison between group I and II; b- comparison between group II and III; c- comparison between group II and IV and between group I and V
Units: Protein-mg/g tissue

Table 2: Effect of *Ananus comosus* on SOD, CAT and GPx levels in liver of control and experimental animal

Groups	SOD	CAT	GPx
Control	1.18±0.15^{b}	1.77±0.05^{c}	1.05±0.20^{c}
DMBA induced	0.85±0.17^{a}	0.74±0.06^{a}	0.49±0.13^{a}
DMBA+*A.comosus*	1.07±0.19^{b}	1.01±0.05^{b}	0.94±0.12^{bc}
DMBA+Tamoxifen	1.23±0.37^{b}	1.20±0.11^{b}	0.97±0.21^{bc}
A.comosus alone	1.22±0.23^{b}	1.83±0.09^{c}	1.06±0.18^{c}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at p< 0.05 (DMRT)
Comparison between the groups
a- comparison between group I and II; b- comparison between group II and III; c- comparison between group II and IV and between group I and V
Units: SOD - Inhibition of 50% nitrite formation/min/mg protein; CAT - µmole of H_2O_2 consumed/min/mg protein; GPx - µg of glutathione oxidized/min/mg protein

Table 3: Effect of *Ananus comosus* on SOD, CAT and GPx levels in kidney of control and experimental animal

Groups	SOD	CAT	GPx
Control	1.12±0.34^{bc}	2.03±0.11^{d}	1.56±0.27^{d}
DMBA induced	0.82±0.49^{a}	0.87±0.08^{a}	0.64±0.18^{a}
DMBA+*A.comosus*	1.05±0.23^{b}	1.13±0.24^{b}	0.83±0.11^{b}
DMBA+Tamoxifen	1.31±0.52^{c}	1.69±0.17^{c}	1.18±0.17^{c}
A.comosus alone	1.09±0.37^{b}	2.02±0.14^{d}	1.45±0.19^{d}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at p< 0.05 (DMRT)
Comparison between the groups
a- comparison between group I and II; b- comparison between group II and III; c- comparison between group II and IV and between group I and V
Units: SOD - inhibition of 50% nitrite formation/min/mg protein; CAT - µmole of H_2O_2 consumed/min/mg protein; GPx - µg of glutathione oxidized/min/mg protein

Table 4: Effect of *Ananus comosus* on GR, GST and G6PD levels in liver of control and experimental animal

Groups	GR	GST	G6PD
Control	11.25±0.82^{c}	73.07±2.41^{c}	13.09±0.14^{c}
DMBA induced	5.00±0.77^{a}	26.43±2.4^{a}	8.55±0.20^{a}
DMBA+*A.comosus*	8.25±0.82^{b}	54.42±2.38^{b}	10.23±0.51^{b}
DMBA+Tamoxifen	9.25±1.13^{b}	73.74±2.4^{c}	11.07±0.22^{b}
A.comosus alone	10.25±1.13^{c}	72.30±2.55^{c}	13.00±0.12^{c}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at p< 0.05 (DMRT)
Comparison between the groups
a- comparison between group I and II; b- comparison between group II and III; c- comparison between group II and IV and between group I and V
Units: GR - µmole of glutathione utilized/min/mg protein; GST - µmoles of CDNB – GSH conjugate formed/min/mg protein; G6PD - nmoles of $NADP^{+}$ reduced/min/mg protein

Table 5: Effect of *Ananus comosus* on GR, GST and G6PD levels in kidney of control and experimental animal

Groups	GR	GST	G6PD
Control	9.99±0.77^{d}	45.09±2.41^{c}	11.28±0.49^{b}
DMBA induced	2.75±1.13^{a}	21.77±2.40^{a}	9.02±0.14^{a}
DMBA+*A.comosus*	5.99±0.95^{b}	32.65±2.95^{b}	10.47±0.56^{ab}
DMBA+Tamoxifen	8.00±0.76^{c}	43.53±4.82^{c}	11.08±0.08^{b}
A.comosus alone	8.99±1.90^{c}	46.64±4.17^{c}	11.34±0.19^{b}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at p< 0.05 (DMRT)
Comparison between the groups
a- comparison between group I and II; b- comparison between group II and III; c- comparison between group II and IV and between group I and V
Units: GR - µmole of glutathione utilized/min/mg protein; GST - µmoles of CDNB – GSH conjugate formed/min/mg protein; G6PD -nmoles of $NADP^{+}$ reduced/min/mg protein

Table 6: Effect of *Ananus comosus* on non-enzymic antioxidant levels in liver of control and experimental animal

Groups	GSH	Vitamin C	Vitamin E
Control	17.36±0.36^{c}	1.99±0.43^{c}	1.58±0.05^{c}
DMBA induced	5.07±3.16^{a}	0.76±0.07^{a}	0.93±0.09^{a}
DMBA+*A.comosus*	10.95±0.68^{b}	1.33±0.20^{b}	1.43±0.14^{b}
DMBA+Tamoxifen	12.98±0.51^{b}	1.57±0.20^{b}	1.48±0.35^{c}
A.comosus alone	16.23±1.15^{c}	2.09±0.44^{c}	1.56±0.36^{c}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at p< 0.05 (DMRT)
Comparison between the groups
a- comparison between group I and II; b- comparison between group II and III; c- comparison between group II and IV and between group I and V
Units: GSH, Vitamin C, Vitamin E-µg/mg protein

Table 7: Effect of *Ananus comosus* on non-enzymic antioxidant levels in kidney of control and experimental animal

Groups	GSH	Vitamin C	Vitamin E
Control	11.77±0.68^{c}	1.93±0.41^{c}	3.55±0.36^{b}
DMBA induced	3.92±0.61^{a}	0.91±0.13^{a}	2.31±0.17^{a}
DMBA+*A.comosus*	7.03±0.84^{b}	1.04±0.09^{b}	3.43±0.28^{b}
DMBA+Tamoxifen	8.79±1.19^{b}	1.30±0.18^{b}	3.49±0.24^{b}
A.comosus alone	11.76±0.85^{c}	1.90±0.21^{c}	3.57±0.61^{b}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at p< 0.05 (DMRT)
Comparison between the groups
a- comparison between group I and II; b- comparison between group II and III; c- comparison between group II and IV and between group I and V
Units: GSH, Vitamin C, Vitamin E-µg/mg protein

Table 8: Effect of *Ananus comosus* on lipid peroxidation level in liver and kidney of control and experimental animal

Groups	Liver	Kidney
Control	15.22±0.26^{a}	9.76±0.31^{a}
DMBA induced	30.92±0.30^{b}	16.37±0.24^{b}
DMBA+*A.comosus*	18.82±0.15^{a}	12.64±0.40^{ab}
DMBA+Tamoxifen	16.86±0.22^{a}	13.52±0.33^{ab}
A.comosus alone	15.52±0.20^{a}	9.56±0.21^{a}

Values are expressed as mean ± SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at p< 0.05 (DMRT)
Comparison between the groups
a- comparison between group I and II; b- comparison between group II and III; c- comparison between group II and IV and between group I and V
Units: LPO - nM of MDA formed/min/mg protein

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ASYMBIOTIC SEED GERMINATION AND SYNTHETIC SEED PREPARATION OF *VANDA TESTACEA* (LINDL.) RCHB.F .

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ABSTRACT

A protocol for asymbiotic seed germination and synthetic seed preparation of *Vanda testacea*(Lindl.) Rchb.f. from mature pods was developed. Seed germination was successfully established on half strength B_5 (Gamborg's medium) supplemented with 1.0mg/L GA_3. Protocorm like bodies (PLB's) were formed after 90 days of culture and further artificial seed preparation of matured protocorm was successful using 5% sodium alginate and 100mM calcium chloride. The beads were inoculated on half strength B_5 medium supplemented with 1.0mg/L GA_3.

Keyword: *Vanda testacea*, seed germination, artificial seed preparation, Protocorm.

1. INTRODUCTION

Vanda testacea (Lindl.) Rchb.f. is a species of orchid occurring from the Indian subcontinent to Indochina at the elevations of 500 to 2000 meters. It is an epiphytic perennial. In India orchids form 9% of flora and are the largest family among higher plants. It is estimated that about 1,300 species (140 genera) of orchids are found in our country with Himalayas as their main home and others scattered in eastern and Western Ghats. *Vanda testacea* (Lindl.) Rchb.f. has medicinal properties in sciatic nerve transaction (axotomy) induced peripheral neuropathy in rats (Santh Rani Thaakur and Swapna Pokkula, 2013). Root used to treat nervous disorders, piles, and inflammations as well as a potential anticancerous drug. Leaves, flowers in powder form are used as herbal medicines to cure rheumatism and bronchitis (Chauhan , 1990) . Asymbiotic seed germination by *in vitro* culture, which was first introduced and it has revolutionized the concept of orchid cultivation. Soumi Neha (2013).

Vanda testacea (Lindl.) Rchb.f. has a low rate of multiplication under natural/greenhouse conditions and like other monopodial orchids, surviv al of mother plant is not conducive to a shoot tip / meristem based micropropagation system. It is thus necessary to device a rapid and efficient micropropagation. Orchid seeds became necessary for future sustainable harvesting system and maintaining orchid species to prevent from genetic erosion. Hence the present study was focused on seed germination and artificial seed preparation of *Vanda testacea* using matured pods.

2. MATERIALS AND METHODS

2.1. Source of plant material

The mature pods of *Vanda testacea*(Lindl) Rchb.f. was provided by Dr.T.Muthukumar, Department of Botany, Bharathiar University, Coimbatore.

2.2. Explant selection and mode of sterilization

Collected mature pods were surface sterilized by immersing in 70% ethanol for 10 sec. The sterilized pods were then washed 4-5 times with sterile double distilled water. Further capsules were cut longitudinally with a sterile scalpel and the seeds were inoculated on to half strength B_5 medium.

2.3. Method of media preparation

Half B_5 (Gamborg's) medium was employed in the present study and the composition of the medium is given in Table-1. For media preparation, only analytical reagents of "Hi-media" chemicals and Borosil glassware's were used. Double distilled water was used for media preparation. Stock solutions were prepared separately for macronutrients, micronutrients, iron, potassium iodide and vitamins. All the chemicals were weighed accurately in electronic weighing machine. All the stock solutions were poured in to well stoppered sterilized bottles and preserved in a refrigerator at 4°C. Specific quantity of the stock solutions and growth regulators were pipetted on to a little beaker. Required source, other organic supplements and complex additives (optional) were added. The final volume was made up with distilled water and the pH was adjusted to 5.8 with either 1N NaOH or HCl using a pH meter (ELICO).

To the above said media, 0.8% agar (extra pure gelling point 32-35◦C, Hi media, Bombay) was added, melted in a water bath and the medium was dispensed into culture bottles. The bottles after covering were autoclaved at 1.06 kg pressure/sq cm

for about 20min at 121°C.The autoclaved medium in the culture bottles were allowed to cool. The inoculation was done after 5 days to ensure that the bottles were free from contamination.

Table 1. Composition of Gamborg's (1968) medium

S. no.	Component	mg/l
MAJOR SALTS		
1	$Na_2HPO_4.2H_2O$	150
2	KNO_3	250
3	$CaCl_2.2H_2O$	150
4	$MgSO_4.7H_2O$	250
5	$(NH_4)_2SO_4$	134
MINOR SALTS		
6	KI	0.75
7	H_3BO_3	3
8	$MnSO_4.4H_2O$	10
9	$ZnSO_4.7H_2O$	2
10	$Na_2MO_4.2H_2O$	0.25
11	$CuSO_4.5H_2O$	0.025
12	$CoCl_2.6H_2O$	0.025
13	$C_{10}H_{12}N_2O_8$ Fe Na	43
VITAMINS AND ORGANICS		
14	Meso-Inositol	100
15	Nicotinic acid	100
16	Pyridoxine HCl	100
17	Thiamine HCl	100
18	Cystine	10
19	Sucrose	20
20	Agar	8
21	Ph	5.8

2.4. Growth regulator and it's preparation

Growth regulator like gibberillic acid was used in the experiments.

2.5. Preparation of Gibberillic acid (GA_3)

The stock solution was prepared by dissolving 10 mg of GA_3 in 1mL of ethanol and the volume was made up to 100mL with sterile distilled water. This was used in two different concentrations (0.5 and 1.0 mg/L).

2.6. Culture conditions

The cultures were maintained at 25±2◦C under a 16hr photoperiod of 50-60µmol flux intensity provided by cool white fluorescent tubes.

2.7. In vitro seed germination

Half B_5 (half strength Gamborg's medium) medium was used for seed germination.

2.8. Artificial seed preparation

For encapsulation purpose 5% sodium alginate and 100mM calcium chloride (w/v) were prepared using sterile distilled water.

2.9. Formation of beads

The protocorms were transferred to the sodium alginate solution. The protocorms along with sodium alginate matrix were dropped into a solution of calcium chloride and maintained for atleast 15 min to polymerize the beads. (When sodium alginate drops come in contact with calcium chloride solution surface complexation begins and form round beads). The beads were recovered by discarding the calcium chloride solution and later washed twice with sterile distilled water.

3.0.Culture medium and conditions

The encapsulated protocorms were cultured on a half strength B_5 medium supplemented with 1.0 mg/L of GA_3. All cultures were maintained in the culture room at 25±2°C under a 16hr photoperiod.

4. RESULTS AND DISCUSSION

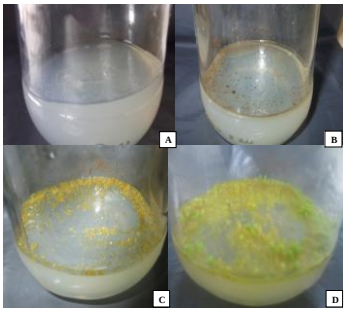
Orchids are faced with habitat destruction pressures due to extensive collections in the past. As a consequence the species has become rare and is restricted to very narrow pockets in its natural habitats (Kaur and Bhutani 2009). Thus to conserve this orchid from extinction and to increase the population size, plant tissue culture and micropropagation can play a significant role (Wochok, 1981). The increasing demand, and export values of orchids each year is driving the expansion of orchid growing areas (Kasikorn Research Center, 2008). A Significant number of identical clones can be raised from a single leaf through direct or callus mediated organogenesis (Arditti, 1977). Many reports have been made in growing the orchid pods in full strength MS medium and Knudson's medium. So the present study mainly focused on growing the seeds on half B_5 medium and to prepare synthetic seeds from protocorms.

The seeds of orchids produced in large numbers in each capsule are highly fragile and possess virtually no stored food material and endosperm. The epiphytic orchid *V.testace* has poor seed germination under natural condition. Seeds were cultured on Knudson's C medium, enriched with various concentrations of organic additives and plant growth regulators to study asymbiotic germination, seedling development and optimization of the cultural requirements (Mukhopadhyay and Roy, 1994).

The matured capsules are brown and thick in nature. The surface sterilized matured pods were opened under aseptic condition and the seeds were deposited on half strength B_5 (Half Strength Gamborg's Medium) medium supplemented with

1.0mg/L GA_3. After 45 days of inoculation the seeds started germinating. Further the germinated seeds were allowed to grow in the same medium up to 90 days. Protocorm like bodies (PLB's) were formed after 90 days of culture (Plate-1). Seed germination and development of three *Vanda* hybrids were reported by Timothy Johnson (2007). Six asymbiotic orchid seed germination media were examined for their effectiveness in promoting germination and subsequent protocorm development of *Bletia purpurea* seeds was reported by Malmgren (1996).

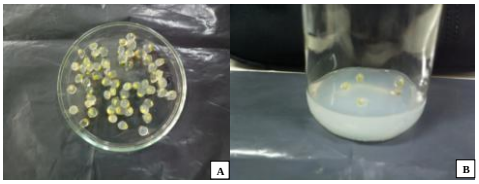
Plate-1



A. Inoculated orchid pod
B. Changes in colour after 30 days of inoculation
C. Formation of protocorms after 45 days of inoculation
D. Well grown protocorms after 60 days of inoculation

Further the fully developed protocorm like bodies (PLB's) were separated from the medium and subjected to artificial seed preparation. The mature protocorm like bodies (PLB's) were collected and mixed with 5% sodium alginate and dropped into 100mM of calcium chloride solution to form beads of synthetic seeds. Subsequently the beads were washed with sterile double distilled water and inoculated on half strength B_5 medium supplemented with 1.0mg/L GA_3(Plate-2). The seedling development of *Onicidium sp*. was best on the MS medium supplemented with 2mg/l BA (Sharma, 1991). Several valuable species of *Dendrobium* have been reported to be propagated through asymbiotic germination via immature seeds (Vij *et al.,* 1981).

Plate-2



A. Prepared synthetic seed
B. Synthetic seed inoculated on half B_5 +1mg/L GA

5. CONCLUSION

The findings of present study suggested that the asymbiotic seed germination was best on half B_5 medium supplemented with 1.0mg/L GA_3 and 5% sodium alginate and 100mM of calcium chloride was suitable for artificial seed preparation.

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PREPARATION AND STANDARDIZATION OF BIOCOMPATIBLE BACTERIAL CONSORTIUM FOR THE ENHANCED GROWTH OF CROP PLANTS

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ABSTRACT

Intensive application of agrochemicals leads to several agricultural problems and poor cropping systems. The extensive research program on beneficial bacteria and fungi has resulted in the development of a wide range of bio-fertilizers, which satisfied the nutrient requirements of crops and increased the crop yield as well.In the present study;variousPlant Growth Promoting Rhizobial Bacteria from the 6 soil samples were isolated, characterised and stored. PGPR- Biofertilizer was prepared by mixing isolated PGPR-BACTERIA with various compounds. Seeds of Bengal gram,Green gram and Chicken peas were collected and treated with biofertilizer; controls (without biofertilizers) also were maintained for comparison. Biofertilizers (*Azotobacter, Pseudomonas, Enterobacter,Bacillus, Phosphobacteria* and *Rhizobium)* were used alone as well as in combination of all inoculates. Seeds were sown in cups and grown in three batches. The growth and yield parameters of those plants such as length of root and stem, leaves count, total chlorophyll, carotenoid and protein content were observed.The total chlorophyll and carotenoid content in plants were estimated. High chlorophyll, carotenoid and protein content were observed in Rhizobium inoculants when compared with other inoculants. High chlorophyll content was observed in Chicken peas and high carotenoid content in Green gram and Chicken peas. High chlorophyll was observed in Chicken peas. High protein content was observed in T5 and low protein content was observed in T2 when compared with other inoculants, Chicken pea showed high protein content and Bengal gram showed low protein content.

Key words: Biofertilizer, Bacterial Consortium, *Rhizobium.*

1. INTRODUCTION

A biofertilizeris a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Singh *et al*., 2008).Bio-fertilizers provide eco-friendly organic agro-input and are more cost-effective than chemical fertilizers. Bio-fertilizers such as *Rhizobium, Azotobacter, Phosphobacterium, Pseudomonas* and *Bacillus*, have been in use a long time. *Rhizobium* inoculants are used for leguminous crops. *Azotobacter* can be used with crops like wheat, maize, mustard, cotton, potato and other vegetable crops. *Pseudomonas* inoculations are recommended mainly for sorghum, millets, maize, sugarcane and wheat. *Rhizobium* fixes atmospheric nitrogen and are used as inoculations for paddy crop grown both under upland and low-land conditions.Other types of bacteria, so-called phosphate-solubilising bacteria, or *Pseudomonas putida* P13 (Vijila*et al*., 2008) are able to solubilise the insoluble phosphate from organic and inorganic phosphate sources (Abbas*et al*.,2013).

Bengal gram is the third important pulse crop in India. It is annual pulse crop and native to central Asia. It is also extensively grown in West Indies, Japan and other tropics/sub-tropical countries. Bengal gram seeds are highly nutritious containing higher amount of protein (24 to 26%) and are reported to be rich in potassium, phosphorous and calcium with good amount of sodium. Green gram is one of important pulse crop in India. Commonly called as mung beans are occasionally used in Indian cuisine; beans without skins are boiled to make dry preparation often served with rice. Chicken pea is one of the earliest cultivated legumes: 7,500- year old remains have been found in the Middle East. Chicken peas are known for their medical uses such as increasing sperm and milk, provoking menstruation and urine and helping to treat kidney stones.

2. MATERIALS AND METHODS

2.1.Isolation and Identification of Microorganisms

Soil samples were collected andsubjected to serial dilution with dilution factor 1:10 and bacteria's were grown on nutrient agar using spread plate method. After that, different bacteria's were grown on their selective media's and then streaked on nutrient agar plates to obtain pure cultures.

2.2.Preparation of Biofertilizer

The compounds (given in table) were mixed with all isolated cultures (5ml each) in a beaker

under sterile conditions to prepare PGPR biofertilizer.

Table1. Composition of biofertilizer

S.No	Compounds	Weight (g/30ml)
1.	Dolomite Talc	100g
2.	Carboxy Methyl Cellulose	1g
3.	Calcium Carbonate	1g
4.	Crab Shell Powder	0.05g
5.	Barley Powder	0.05g

2.3. Determination of Plant Growth Using MICR the Plants

Seeds of Bengal gram, Green gram and Chicken peas were collected. Seeds were treated with biofertilizers and controls (without biofertilizers) also were used as reference. The microbial consortium consists of *Azotobacter, Pseudomonas, Enterobacter, Bacillus, Phosphobacterium* and *Rhizobium* were used alone as well as in combination of all inoculates. Seeds were sown in cups. The plants were grown in three batches. The batch –I includes inoculants such as T0-control (without any microbial inoculation), T1-*Rhizobium*,T2- *Pseudomonas*, T3- *Phosphobacteria*, T4-*Bacillus*,T5- *Enterobacter*, T6- *Azotobacter* and T7- *Rhizobium*+*Pseudomonas* + *Phosphobacteria* + *Bacillus* + *Entero* + *Azotobacter.* The batch –II includes biofertilizer powder mixed with seeds. The batch –III includes soil mixed with bio fertilizer powder. From each entry, 10 plants were randomly selectedfor recording observations on important yield attributing characters, plant height,and length of the root, leaves count, estimation of total chlorophyll, carotenoid and protein content during the plant growth period.

2.4. Estimation of Plant Chlorophyll

About 0.5 mg of fresh leaf was ground in a mortar and pestle with 20 ml of 80 per cent acetone. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was saved. The pellet was restricted with 5 ml of 80 per cent acetone each time, until it become colourless. All the supernatants were pooled and utilized for chlorophyll determination. Absorbance was measured at 645 and 663nm in spectrophotometer. The chlorophyll content was determined by using the following formulae.

Chlorophyll 'a' (mg/g fr. wt.) = $(0.0127) \times (OD_{663}) - (0.00269) \times (OD_{645})$

Chlorophyll 'b' (mg/g fr. wt.)

$= (0.229) \times (OD_{645}) - (0.00488) \times (OD_{663})$

Total chlorophyll(mg/g fr. wt.)

$= (0.0202) \times (OD_{645}) - (0.00802) \times (OD_{663})$

2.5. Estimation of Plant Carotenoid

The same chlorophyll extract was measured at 480nm, in spectronic-20 to estimate the carotenoid content.

Carotenoid (mg/g fr. wt.) = D×F×V×10/wt. ×2500

$= (OD_{480}+OD_{114})$ '(OD_{663})-$(OD_{638}$ 'OD $_{645})$

2.6. *Estimation of Plant Protein (Lowry et al)*

About 0.5 mg of plant materials was macerated with a pestle and mortar with 10 ml of 20 per cent trichloroacetic acid. The homogenate was centrifuged for 15 min at 600 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 ml of 0.1 N NaOH. This extract was used for the estimation of protein. From this extract, 1 mL of sample was taken in a 10 mL test tube and 5 mL of reagent was added. The solution was mixed well and kept in dark for 10min. Later 0.5 mL folinphenol was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in the Spectronic-20. Blank prepared without protein sample was used for zerosetting.

3. RESULTS AND DISCUSSION

In the present investigation, the rhizosphere soil samples were collected from crop plants, and it was subjected to isolate the rhizospheric bacteria by standard methods, all the rhizospheric bacteria were used to prepare the effective microbial consortia as plant growth promoting agent for crop plants.

3.1. Isolation and Identification of Microorganisms

The various PGPR-BACTERIA'S were isolated from the soil and their characteristics were studied using selective medium and pure cultures were stored. There was significant number of different bacterial isolates were obtained and enriched for making biomass, which is listed below (Table 2). The preliminary tests and growth characteristics on respective selective medium supports to precede the identification of PGPR. Further, the biochemical profiles evidenced for naming the isolated bacteria at the genus level with reference to the Bergey's manual for bacterial identification.

Table 2. Growth characteristics of rhizosphere bacteria

S.No.	Bacteria	Selective Media	Characteristics
1.	*Pseudomonas* sp.	King's-B Agar	Fluorescent colonies were observed.
2.	*Enterobacter* sp.	Violet Red Bile Glucose Agar	Red coloured colonies were observed.
3.	*Rhizobium* sp.	Yeast Mannitol Agar	White colonies along with air bubbles (gas production).
4.	*Phosphobacterium* sp.	Nutrient Agar	White colonies were observed.
5.	*Bacillus* sp.	Nutrient Agar	Zone of hydrolysis(iodine test) was observed
6.	*Azotobacter* sp.	*Azotobacter* medium	White colonies were observed

3.2. Determination of Plant Growth by the Activity of PGPR

The growth and yield parameters of Bengal gram, Green gram and Chicken peas such as plant height, root length, leaves count, total chlorophyll, carotenoid and protein content were significantly increased by PGPR application in all concentrations when compared to control plants.

Utilization of biological fertilizer increased fresh and dry weight; no. of pods per plant that it could be due to increasing other nutrient absorption, also biological phosphate fertilizer can be used as a solution for increasing phosphate and micronutrient absorption in the alkaline soil. Both qualitative and quantitative characteristics were significantly increased by phosphate-solubilising microorganisms and also increased the growth and resistance of plants in water deficit conditions (Selvakumar *et al.,* 2012).

The utilization of phosphate-solubilising microorganisms, account for about 45% of the total biofertiliser production and use. This bacterium helps in increasing crop productivity by way of helping in solubilisation of insoluble phosphorus, stimulating growth by providing hormones, vitamins and other growth factors. The availability of phosphorus to legume crop is a key constraint to its production (Laemmli *et al*., 1970).

Several genera of rhizobacteria belonging to *Pseudomonas* spp. And *Bacillus* spp. are reported to solubilise zinc. Microbes solubilise the metal forms by protons, chelated ligands, and oxido reductive systems present on the cell surface and membranes (Crane *et al.,* 1985; Hughes and Poole, 1991; Wakatsuki, 1995). These bacteria also exhibit other traits beneficial to plants, such as production of phytohormones, antibiotics, siderophores, vitamins, antifungal substances, and other cellular components (Rodr´ıguez and Fraga, 1995) Microbes are potential alternate that could cater plant nutrient requirement by up taking the energy materials from the soil.

The soil microorganisms are responsible for transfer of the immobilized soil phosphorus into available form through which phosphorus becomes easily available to these plants. Co-inoculation can benefit plant growth by different mechanisms. However one of the most commonly reported plant growth promotion mechanism by bacteria is the changing of morphological and physiological changes in root system. An increase in the number of lateral roots and root hairs cause addition of root surface available for nutrients and water uptake. Higher water and nutrient uptake by inoculated roots caused an improved water status of plant, which in turn could be the main factor enhancing plant growth (Parvatham *et al*., 1989).

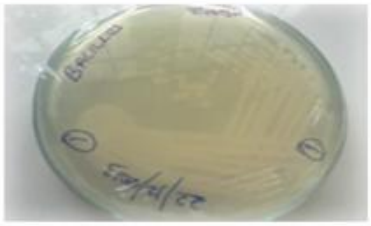
Table 3. Measurement of length of stem, root and No. of leaves Batch- I

Sample		T0	T1	T2	T3	T4	T5	T6	T7
Green Gram	Stem Length (cm)	18.4	20.2	-	23.0	25.3	28.1	18.2	-
	Root Length(Cm)	3.9	6.3	-	4.8	3.9	6.7	6.9	-
	Leaves Count	2	5	-	6	8	12	10	-
Chicken Peas	Stem Length (cm)	16.1	26.3	28.4	28.8	31.7	38.5	21.3	19.2
	Root Length (cm)	3.9	4.3	5.5	5.9	7.9	9.0	5.8	4.4
	Leaves Count	4	7	6	8	8	16	10	6
Bengal Gram	Stem Length (cm)	21.2	18.3	12.7	17.2	19.5	-	16.2	-
	Root Length(cm)	5.2	6.4	5.8	6.2	6.5	-	7.0	-
	Leaves Count	3	10	4	7	5	-	8	-

T0 – Control (without bacterial strain), T1- *Rhizobium*, T2- *Pseudomonas*, T3- *Phosphobacteria*, T4-*Bacillus*, T5- *Enterobacter*, T6-*Azotobacter* and T7- *Rhizobium*+ *Pseudomonas* + *Phosphobacteria* + *Bacillus* + *Enterobacter* + *Azotobacter*

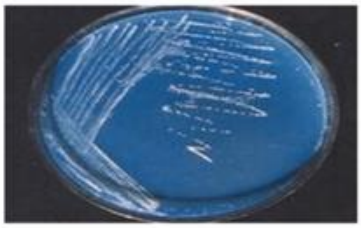
Rhizobium

Azotobacter

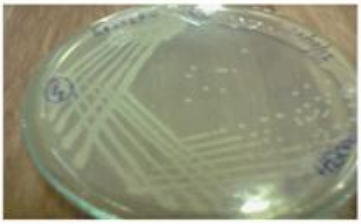


Bacillus

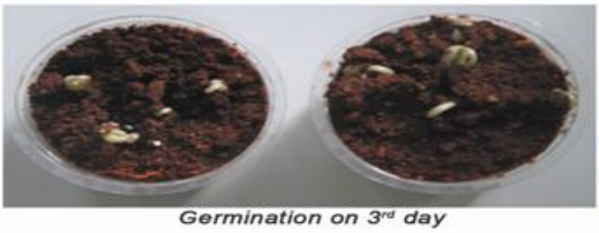
Phosphobacterium



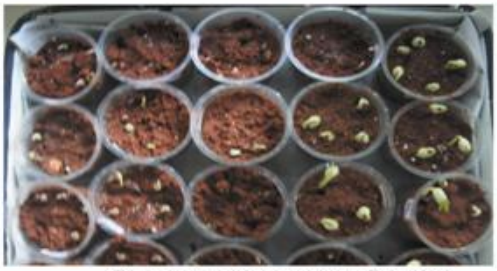
Azospirillum



Enterobacter

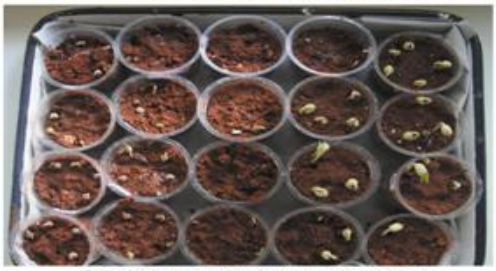


Germination on 3rd day



Plants on pot system-Batch-I

Germination on 5th day



Plants on pot system-Batch-II

Germination on 15th day

GERMINATION IN BATCH-I ON 9th day

GERMINATION IN BATCH-II ON 9th day

GERMINATION IN BATCH-III ON 9th day

Table 4. Plant growth on Batch-II & III

Content	Bengal Gram		Chicken Peas		Green Gram	
	Batch-II	Batch-III	Batch-II	Batch-III	Batch-II	Batch-III
Stem length (cm)	23.4	21.3	32.4	38.6	26.1	28.6
Root length (cm)	12.3	12.2	17.3	19.4	15,3	16.2
Leaves count (No.)	17	19	29	38	24	29

Various root and stem length in three plants

Significant Leaves Count In Chicken Peas

3.3. Measurement of Length of Stem, Root and No. of Leaves

Ten plants were selected from those grown in a randomised block design in three replications and important yield attributing characters like number of leaves, length of the stem and length of root were observed and recorded.

3.4. Estimation of Chlorophyll and Carotenoid

The total chlorophyll and carotenoid content in Batch 1, Batch 2, and Batch 3 was estimated (Table 5, 6, 7). In Batch 1, high chlorophyll content was observed in T1 Chicken peas and high carotenoid content in T5 Green gram, T5 Chicken peas. In Batch 2, high chlorophyll and carotenoid content was observed in Chicken peas. In Batch 3, high carotenoid content was observed in Bengal gram and high carotenoid content in Green gram. The isolate T7 did not showed significant growth of green gram and Bengal gram. All the experiments were done with triplicates.

3.5. Existence of plant protein due to bacteria consortia

The protein content in all batches was estimated. In Batch 1, high protein content was observed in T5 when compared with others and low protein content was observed in T2. In Batch 2 and Batch 3, Chicken pea showed high protein content and Bengal gram showed low protein content.

Table 5 Estimation of Chlorophyll and Carotenoid on Batch - I

Sample (mg/g)		T0	T1	T2	T3	T4	T5	T6	T7
Green Gram	Chlorophyll	1.08	3.4	-	2.67	2.4	2.1	2.78	-
	Carotenoid	0.02	0.02	-	0.04	0.01	0.09	0.03	-
Chicken Peas	Chlorophyll	1.98	3.45	3.23	3.11	2.98	2.94	2.67	1.01
	Carotenoid	0.09	0.03	0.05	0.03	0.07	0.09	0.02	0.01
Bengal Gram	Chlorophyll	0.90	2.56	2.3	2.90	2.08	-	2.67	-
	Carotenoid	0.03	0.02	0.06	0.03	0.45	-	0.89	-

T0 – Control (without bacterial strain), T1- *Rhizobium*, T2- *Pseudomonas*, T3- *Phosphobacteria*, T4-*Bacillus*, T5- *Enterobacter*, T6-*Azotobacter* and T7- *Rhizobium+ Pseudomonas + Phosphobacteria + Bacillus + Enterobacter + Azotobacter*

Table 6. Estimation of Chlorophyll and Carotenoid on Batch – II & III

Sample (mg/g)	Bengal Gram	Chicken Peas	Green Gram
Chlorophyll	2.02	2.98	1.92
Carotenoid	0.56	0.89	0.71

Table 7. Estimation of Chlorophyll and Carotenoid on Batch - III

Sample (mg/g)	Bengal Gram	Chicken Peas	Green Gram
Chlorophyll	2.95	2.91	2.04
Carotenoid	0.45	0.55	0.78

Table 8. Estimation of protein on Batch-I.

Protein	T0	T1	T2	T3	T4	T5	T6	T7
Green gram	3.09	3.23	-	3.31	3.22	3.98	3.20	-
Chicken peas	3.45	3.67	3.34	3.12	3.76	3.44	3.71	3.33
Bengal gram	3.00	3.21	3.09	3.45	3.81	-	3.27	-

T0 – Control (without bacterial strain); T1- *Rhizobium*, T2- *Pseudomonas*, T3- *Phosphobacteria*, T4-*Bacillus*, T5- *Enterobacter*, T6-*Azotobacter* and T7- *Rhizobium+ Pseudomonas + Phosphobacteria + Bacillus + Enterobacter + Azotobacter*

Table 9. Estimation of protein on Batch-II & III

Protein	Bengal Gram	Chicken Peas	Green Gram
Batch –II	3.11	3.56	3.28
Batch –III	3.49	3.89	3.71

The present study clearly demonstrated that inoculation with plant growth promoting Rhizobacteria significantly enhanced the growth of Green gram in all dimensions. In this study application of microbial consortia in the preparation of biofertilizer has increased the total biomass of plants include root, stem and leaf count. Also it is showed that PGPR inoculation effectively increases surface area of roots and root weight. The earlier study also showed the similar findings (Cakmakc *et al.,* 2007). The variation in enhancement of plant growth by these strains may be due to the difference in the quantity of Chlorophyll and Carotenoid induced by each strain on the respective crop plant batches.

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PHYTOCHEMICAL STUDIES ON THE TERPENOIDS OF MEDICINALLY IMPORTANT PLANT *SOLANUM VILLOSUM* (MILL.) USING HPTLC

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ABSTRACT

To determine terpenoid profile of *Solanum villosum* using high performance thin layer chromatography (HPTLC) technique. 2cl of test solution and 2cl of standard solution was loaded as 6mm band length in the 3 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The extract was run along with the standard terpenoid compound and it was observed that the extract showed the presence of terpenoid and it was confirmed from the chromatogram after derivatization. The Rf value of the different compounds present in the extract was found to 0.04,0.21,0.27,0.55,0.59,0.65,0.75,0.80,0.90 and 0.94 of peak 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively. Among them, peaks 2, 6, 7 and 10 were found to be terpenoid compounds. It can be concluded that HPTLC analysis of ethanolic leaf extract of *Solanum villosum* (Mill) can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

Key words: HPTLC, ethanol extract, *Solanum villosum*, phytochemicals, terpenoid.

1. INTRODUCTION

Solanum villosum (Mill.) belongs to family Solanaceae, it is commonly known as red-fruit nightshade, is widely distributed in many parts of India. *Solanum* is one of the most important and largest genera of the family Solanaceae comprising of about 84 genera and 3000 species were identified throughout the worldwide. The plant is an ayurvedic herb with multiple medicinal properties (Nandita Chowdhury *et al*., 2008).

The plant *Solanum villosum* contain many primary and secondary metabolites such as, alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, steroids, carbohydrates, glycosides, amino acids and proteins. The genus of *Solanum* species contains excellent antioxidant properties and free radical scavenging ability (Annie Jacob and Radha, 2013).

The plants of *S. nigrum* complex has been traditionally used as an analgesic, antispasmodic, antiseptic, antidysentric, antinarcotic, emollient, diuretic, tonic, soporific, laxative, anticancer, antiulcer and for disorders of neuro-vegetative system etc. (Edmonds and Chweya, 1997). This medicinal value is mainly attributed to the alkaloid content of the plants.

The plant compounds mainly used for treating worms, cold, hoarseness of voice, fever, dysuria, enlargement of the liver, muscular pain, spleen and stone in the urinary bladder (Akilesh Sharma *et al*., 2011; Watt and Breyer-Brandwijk 1962; Aslanov and Novruzov 1978).

In the present study, HPTLC analysis of ethanolic leaf extract of *Solanum villosum* specifically for terpenoid profile was compared with the standards. The *Solanum villosum* and related species are widely used as leafy herbs and vegetables, as a source of fruit and for various medicinal purposes. In spite of known uses in traditional medicines, no documented evidence is available on terpenoid compound analysis. So the HPTLC analysis is to provide information for terpenoid content of the plant extract.

2. MATERIALS AND METHODS

2.1. Plant material

The leaves of the *Solanum villosum* (Mill.) plant were collected from Thadagam hills at Coimbatore district, Tamilnadu, India. The specimen sample was authenticated by Dr.V.S.Ramachandran, Associate Professor, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India. The voucher specimen was deposited in the herbarium center, Department of Botany, Bharathiar University, Coimbatore.

2.2. Extraction of plant material

Plant materials thoroughly washed and shade dried at room temperature after that grind into powder was packed with No.1 Whatman filter paper and placed in soxhlet apparatus along with ethanol. The crude extract were collected and dried at room temperature, 30˚C after which yield was weighed and then performed.

2.3. HPTLC analysis of ethanolic extract of Solanum villosum (Mill)

Test solution preparation: The given ethanol extract 100mg was weighed in an electronic balance (Afcoset) and dissolved in 1ml ethanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

2.4. Sample application

2cl of test solution and 2cl of standard solution was loaded as 6mm band length in the 3 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

2.5. Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Terpenoid) and the plate was developed in the respective mobile phase up to 90mm.

2.6. Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV366 nm.

2.7. Derivatization

The developed plate was sprayed with respective spray reagent (Terpenoid) and dried at 100°C in Hot air oven. The plate was photo-documented in Day light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. Scanning Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4 version.

2.8. Analysis details for Terpenoid

Mobile phase: n-Hexane - Ethyl acetate (7.2 : 2.9). Spray reagent: Anisaldehyde sulphuric acid reagent.

3. RESULTS AND DISCUSSION

3.1. HPTLC analysis of Terpenoids

3.1.1. Detection

Blue, bluish violet coloured zones at Visible light mode present in the given standard and sample track observed in the chromatogram after derivatization, which confirmed the Presence of Terpenoid in the given standard and maybe in sample.

The ethanolic leaf extract of *Solanum villosum* was run along with the standard terpenoid compound and it was observed that the extract showed the presence of terpenoid and it was confirmed from the chromatogram after derivatization. The Rf value of the different compounds present in the extract was found to 0.04, 0.21, 0.27, 0.55, 0.59, 0.65, 0.75, 0.80, 0.90 and 0.94 of peak 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively. Among them, peaks 2, 6, 7 and 10 were found to be terpenoid compounds.

The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards (WHO, 1998). Modern chromatographic techniques like HPLC and HPTLC were used to judge the authenticity of traditional recommendations (Khan *et al*., 2009). The HPTLC method can be used for phytochemical profiling of plants and quantification of compounds present in plants, with increasing demand for herbal products as medicines and cosmetics, there is an urgent need for standardization of plant products (Pawar *et al*., 2010).

HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug (Mauji *et al*., 2011). HPTLC finger printing profile is useful as phytochemical marker and also a good estimation of genetic variability in plant populations. Thus the HPTLC fingerprint profiles of the major chemical constituents in the crude extract along with their Rf values and percentage proportions were recorded which would serve as a reference standard for the scientist who engaged in research on the medicinal properties of this plant (Johnson *et al*., 2011).

The phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques. In the last two decades, HPTLC has emerged as an important tool for the qualitative, semiqualitative and quantitative phytochemical analysis of herbal drugs and formulations. The major advantage of HPTLC is that several samples can be analysed simultaneously using a small quantity of mobile phase (Modi *et al*., 2008). HPTLC fingerprint profiles of the *Solanum villosum* leaf extract for terpenoids have been developed. Rf values and the relative percentage of the separated compounds were recorded.

Table 1. HPTLC analysis of ethanolic leaf extract of *Solanum villosum* for Terpenoid profile

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.85	90.8	3196.5	Terpenoid standard
Sample A	1	0.04	541.7	13312.1	Unknown
Sample A	2	0.21	27.1	601.7	Terpenoid 1
Sample A	3	0.27	85.7	2998.0	Unknown
Sample A	4	0.55	11.4	112.9	Unknown
Sample A	5	0.59	31.1	1464.8	Unknown
Sample A	6	0.65	15.3	270.2	Terpenoid 2
Sample A	7	0.75	66.0	1939.4	Terpenoid 3
Sample A	8	0.80	65.9	2166.5	Unknown
Sample A	9	0.90	26.9	868.9	Unknown
Sample A	10	0.94	21.4	459.8	Terpenoid 4

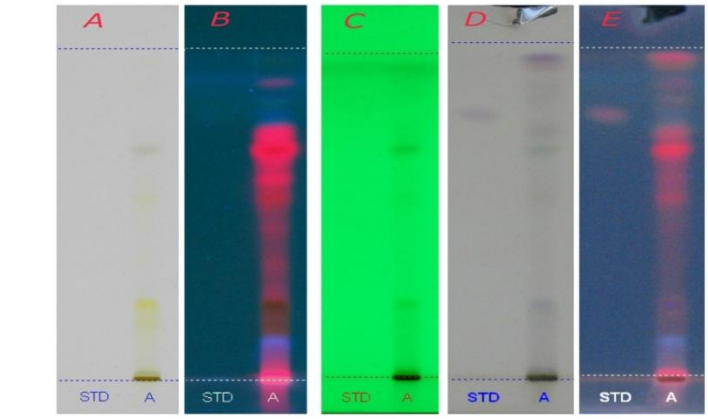

Figure 1. HPTLC studies on the terpenoid of medicinally important plant *S. villosum*

A: HPTLC of the ethanolic leaf extract of *S. villosum* under daylight. **B:** HPTLC of the ethanolic leaf extract of *S. villosum* under UV 366 nm **C:** HPTLC of the ethanolic leaf extract of S. villosum under UV 254 nm. **D:** HPTLC of the ethanolic leaf extract of *S. villosum* under daylight – after derivatization. **E:** HPTLC of the ethanolic leaf extract of *S. villosum* under UV 366 nm after derivatization.

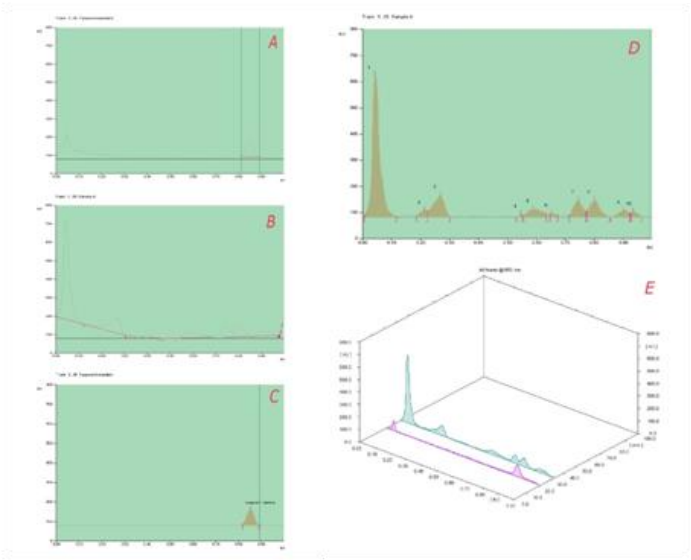

Figure 2. HPTLC chromatogram of ethanolic leaf extract of *S. villosum* (Mill.)

A: HPTLC chromatogram of Track STD – Terpenoid standard Baseline display (Scanned at 366nm), **B:** HPTLC chromatogarm of Track STD – Terpenoid standard Peak densitogram display (Scanned at 366nm), **C:** HPTLC chromatogarm of Track A – Sample A ethanolic leaf extract of *S.villosum* - Baseline display (Scanned at 366nm), **D:** HPTLC chromatogram of Track A – Sample A ethanolic leaf extract *S. villosum* - Peak densitogram display (Scanned at 500 nm), **E:** HPTLC chromatogram of 3D display of all Tracks.

Terpenoids composed of "isoprenoid" units constitute one of the largest group of natural products accounting for more than 40 000 individual compounds, with several new compounds being discovered every year (Sacchettini and Poulter, 1997; Peñuelas and Munné-Bosch, 2005; Withers and Keasling, 2007). Most of the terpenoids are of plant origin; however, they are also synthesized by other organisms, such as bacteria and yeast as part of primary or secondary metabolism. Terpenoids are synthesized from two five-carbon building blocks, i.e., the isoprenoid units. Based on the number of building blocks, terpenoids are classified into several classes, such as monoterpenes (e.g., carvone, geraniol, *d*-limonene, and perillyl alcohol), diterpenes (e.g. retinol and *trans*-retinoic acid), triterpenes e.g., betulinic acid (BA), lupeol, oleanic acid, and ursolic acid (UA), and tetraterpenes e.g., α-carotene, β-carotene, lutein, and lycopene (Rabi and Bishayee, 2009).

The diverse array of terpenoid structures and functions has provoked increased interest in their commercial use. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory, and immunomodulatory properties (Wagner and Elmadfa, 2003; Sultana and Ata, 2008; Shah *et al*., 2008). In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties (Theis and Lerdau, 2003).

4. CONCLUSION

It can be concluded that HPTLC analysis of ethanolic leaf extract of *Solanum villosum* (Mill.) for terpenoid profile can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

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BIOLOGICAL TREATMENT OF YARN DYEING EFFLUENT BY *PSEUDOMONAS* SP.

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ABSTRACT

The untreated effluents were collected from the Yarn dyeing industries at tirupur for the present study.The physico-chemical characteristics like Colour, Odour, Temperature, pH,TDS,TSS,BOD and COD was analysed before treatment.The effluent was treated biologically by Pseudomonas species.After treatment the pollution load reduction was observed significantly and the colour removal also reduced to a maximum level.

Key words: Yarn dyeing industry, biological, Pseudomonas, physico-chemical

1. INTRODUCTION

The Yarn dyeing and textile dyeing industries have a major share in polluting the aquatic bodies as well as lands and the effluents emanating from these industries have imparted colour to the ground water rendering them unsuitable for human consumption. According to Manivasakam (1995), about 50% of the total volume of the effluent from the textile processing is generated only from dyeing units.More than 8000 chemical product are associated with dyeing process (Society for dyers and Colourist,1976).

These effluents from dyeing industries require proper treatment before being let into the aquatic bodies or irrigation fields.The available physico-chemical treatment before methods are expensive and elaborate.Tortora *et al*.(1995) opined that microbial activity can be used to restore or maintain environment quality by biodegradation stimulation.Therefore, in the present investigation methods is attempted.

2. MATERIALS AND METHODS

2.1. Collection of effluent samples

Effluent from the yarn dyeing units at Tirupur was collected and used in this investigation.Soil samples collected from near the effluent flowing canals were used for the culture of the native bacteria. Bacterial strains belonging to pseudomonas spp. were identified from the samples based on the morphological characteristics using the procedures of Cappucino and Sherman (1999) and Kannan (1996).The physico-chemical characteristics of the diluted effluent were analysed following the standard procedures (APHA,1998) and then subjected to 1:1 dilution. After analyzing the physico-chemical characteristics of the diluted effluent, treatment studies were carried out.

2.2. Treatment of effluent by Pseudomonas spp.

To 100 ml of the diluted effluent containing nutrient broth 1 ml of bacterial inoculums was added and the mouth of the conical flask was plugged tightly.The conical flask were agitated in shaker at 120 rpm for 24,48 and 72 h at 37^0C. The treated samples were analysed for physico- chemical characteristics. For colour removal study, OD values of samples were read at 620nm using spectrophotometer. The concentration of dye present in the untreated and treated effluent was calculated referring to the OD value of the standard dyes solution and expressed in mgl^{-1}.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical analysis of effluent

The physico-chemical characteristics of the yarn dyeing effluent and the diluted effluent (1:1 ratio) are presented in Table 1.

The untreated effluent had a mixture of three dyes,Black B,yellow RML and red RB that imparted blackish brown colour.The effluent was odourless and the temperature was 60^0C immediately after collection.The pH, TDS, TSS and COD levels were high and above the permissible limits prescribed limits prescribed by ISI standards for Industrial waste waters into public sewers.

The results of treatment of the effluent with Pseudomonas spp.are tabulated in Table II.By 48 h of the treatment a remarkable reduction in several parameters has been observed and the trend continued upto 72 h treatment.

3.2. Reduction of Pollution load by Pseudomonas spp.

A drastic reduction in pH from 10.7 in the diluted effluent to 7.57 (29.25%) was observed by 24 h.Further decline in pH (7.29) was recorded by 48

h and the same pH was maintained at 72 h. The TDS level came down from 24,200 mgl-1 to 18,160 mgl-1 by 24 h showing a reduction of 24.96% in proportion to the duration of treatment,TDS level showed further reduction in 48 h (15,640 mgl^{-1}) and 72 h (14,000 mgl-1) marking a percentage reduction of 42.15%. Marginal reduction in TSS content was observed by 24th and 48 h (12,800 mgl-1) and 72 h (12,050 mgl^{-1}).

The BOD level at 24 h treatment (150 mgl^{-1}) as observed in the present study may be attributed to the initial utilization of 0_2 by bacterial culture from the medium. By 48 h,however the BOD level was reduced to 136 mgl^{-1} showing 18.07% reduction and by 72 h 126mgl^{-1} with the reduction of 24.10%.

Significant reduction in COD was recorded in these treatments.The COD level in the untreated effluent was 230 mgl^{-1} and this got reduced to 72 mgl^{-1} by 24 h showing 68.70% reduction, 12.0 mgl^{-1} by 48 h and 8.8 mgl^{-1} by 72 h showing a reduction of 94.78% and 96.17% respectively.Maximun reduction of BOD and COD could be obtained by 48 h itself.

Kanekar and Sarnaik (1995) reported that dye industry effluent in treatment with P.alkaligenes remained stable at pH 8.02.A reduction of 51% COD, 82% BOD,74% TDS, 75% phenol and 60% colour in terms of methyl violet was reported in this work.In the present study, a further reduction of pH upto 7.29 and reduction of 42.15% TDS, 6.35% TSS, 24.10% BOD and 96.17% COD was recorded.

3.3. Decolourisation

Biodegradation of dye in the effluent was followed spectrophotometrically.The reduction in the dye concentration was found to be directly proportional to the duration of treatment (Table III). The diluted effluent had a dye concentration of 258.37 mgl^{-1}.This was reduced to 225.07 mgl^{-1} showing a reduced of 47.14% by 24th.Further reduction to 170.10 mg -1(69.63%) was observed in 48 h and thereafter a gradual decline in dye concentration was observed upto 96 h (76.86%).No significant reduction could be recorded beyond this hour. Hu (1994) demonstrated a decolorisation of 37.4% red G,93.2% RBB,92.4% RP2B and 88% V2RP using Pseudomonas luteola within 48 h.Oxspring *et al*.(1996) using microbial consortium of Alcaligenes faecalis and Commamonas acidiverans in a gravel substratum found a decolourisation of 95 % of Ramazol Black B within 48 h.In the present study using Pseudomonas spp., maximum colour removal of 76.83% could be obtained by 96 h.This falls in line with the findings of nigam *et al*.(1996) who reported 70% removal of colour from textile plant effluent after 3 days treatment using a microbial consortium.

4. CONCLUSION

Biological treatment using Pseudomonas spp. in this investigation throws light on effectiveness of this microbe in reducing the pollution load as well as in the decolourisation process.Therefore, micro organisms are increasingly being focused on biological methods for the degradation and elimination of these pollutants in the effluents.

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Table 1. Physicochemical characteristics of yarn dyeing effluent.

Parameters	ISI Standards for Industrial waste water into public sewers	Untreated effluents	50% Diluted Effluents
Colour	-	Brownish black	Brownish black
Odour	-	Odourless	Odourless
Temperature	-	60^0C	32^0C
pH	5.5-9.0	10.9	10.7
Total Dissolved Solids (TDS)	2100	53300	24200
TSS	750	20800	12867
BOD	500	300	166
COD	250	522	230

Table 2. Efficacy of *Pseudomonas* sp. in the reduction of pollution load.

Parameters	Diluted effluent	Treatment duration			Reduction		
		24 h	48 h	72 h	24 h	48 h	72 h
pH	10.7	7.57	7.29	7.29	29.25	31.87	31.87
TDS	24,200	18,160	15,640	14,000	24.96	35.37	42.15
TSS	12,867	12,800	12,800	12,050	0.52	0.52	6.35
BOD	166	150	136	126	9.64	18.07	24.10
COD	230	72	12	8.8	68.70	94.78	96.17

All parameter except pH is expressed in mg-1

Table 3. Efficacy of *Pseudomonas* sp.in colour removal of yarn dyeing effluent.

Duration of treatment	Dye concentration	% Reduction
24 h	225.07	47.14
48 h	170.10	60.05
72 h	129.32	69.63
96 h	98,053	73.55
120 h	112.63	76.86

Dye concentration of untreated effluent - 425.80 mgl-1
Dye concentration of diluted effluent - 258.37 mgl-1