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RESEARCH ARTICLE

DESIGNING COMMUNITY LANGUAGE LEARNING METHOD BASED CLASSROOM ACTIVITIES USING E-LEARNING RESOURCES TO TEACH LISTENING SKILLS AT THE TERTIARY LEVEL

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ABSTRACT

In the current scenario, English plays an inevitable role in all the fields. The learners and teachers of this language understood the need of it and have taken certain steps to learn/teach language effectively using different methods to meet the needs of the society. Only recently, after the outlook of ELT scholars moved towards a blend of methods to suit the unique needs of a particular class and students during the post methods era, This paper is an attempt to design classroom activities based on CLL using E-learning materials to teach listening skills.

Keywords: Community language learning, E-learning, listening skills and classroom activities.

1. INTRODUCTION

The field of teaching is changing day by day. The new methods are used in the classroom to teach students. In olden days, teachers are given importance. Today, students are given importance. Curriculum, syllabus, teaching method, all are based on the needs of students. New ways of teaching are designed and experimented in the classroom to find out the best way to teaching. Technology is also used for developing the quality of education. This also includes teaching English. English Language Teaching also uses technology. They are called Information and Communication Technology (ICT) tools. Computers, computer software, mobile phone, Apps, television, audio players all comes under ICT. After internet was introduced, teachers also use internet to teach English. This approach is called E-learning.(1)

This study aims to design classroom activities using e-learning tools that can be used to teach listening skills. The study also explores the chance of using Community Language Learning (CLL) for the very purpose. The hypothesis of the study is to find out if Community Language Learning (CLL) based classroom activities can be designed using e-learning materials to teach listening skills. We are well aware of approaches and methods of ELT, The Grammar- Translation Method, Direct Method, Audio- Lingual Method, The Situational Language Teaching, Communicative Language Teaching, Humanistic Approaches, Task-based Teaching(2) and so on so forth. In that line humanistic approach directly deals with soft skill of learners more over the method implement in this study CLL categories under this particular approach.

2. COMMUNITY LANGUAGE LEARNING METHOD AND IT SETUP

Humanistic method which focuses on the spoken aspect of learning languages is the Community Language learning (CLL) method. The CLL method was developed by Charles A. Curran, a professor of psychology at Loyola University in Chicago. Under this method, students work together to develop what aspects of a language they would like to learn. This method focuses on the functional aspect of language.

This method is much like counselling: the teacher or the knower helps the students to solve their problems by supporting and encouraging them. In a typical CLL class, a group of eight to twelve students sit in a circle around an audio recording device viz. a tape recorder. There is a general topic for discussion. A student who wishes to say something on the topic, words this to his teacher in his/her mother tongue. The teacher helps the student to express the idea in the target language while other students watch and observe. When the expression is considered, it is recorded. This is then repeated with other students. Then, the dialogue is transcribed on the blackboard and analysed. Other activities or done in the class based on the conversation.

3. DIGITAL, LISTENING SKILL AND COMMUNITY LANGUAGE LEARNING CLASSROOM

Technology and traditional language teaching methods of each course is unique and depends on the students. However, there are a few principles that should be applied to CLL classes: focus on fluency, accuracy, and learner empowerment. A CLL class has the following stages:

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reflexion, recorded conversation, discussion, transcription, and language analysis.

CLL based activities that utilizes E-learning materials can be effective for the simple reason that they do not need external motivation for learning, they are capable of delivering multimedia content that can go a long way in exposing the students to authentic use of English. Community Language Learning is a humanistic method that holds a lot of promise in terms of adapting it in modern classroom situations.(3) This method has to be applied either of four skills classified in English language teaching(ELT), Hence this methodology was tested with all the skills and finalised with the learning skill(4) hence there are lot of attempt trailed over the skill of listening The study to move the focus towards the present or neo listening skill

3.1. E-learning

A learning framework is evaluation of formalized teaching however with the help of electronic devices is thought as E-learning. While instructing is often situated in or out of the classrooms, the use of computers and web makes it a real part of learning. E-learning will likewise be named as a system exchange of skills and data, and therefore the delivery of instruction is created to a considerable variety of receivers at the same or totally different circumstances.

However, with the fast technology innovation in learning it is currently grasped by the majority. The influence of computer and internet tools to cover the students and learning in e-learning platforms, computer, laptops, mobile phones and tablets these gadgets are plays vital role in classrooms learning. In this progress books are getting replaced by electronic educational materials like pen drives or CDs, Students discussion can also be shared via the Internet, which is accessible 24/7, anywhere, anytime.

4. DESIGNING CLASSROOM ACTIVITY BASED ON COMMUNITY LANGUAGE LEARNING METHOD

The task is concern Classroom activity is the final product that the students see in the class. All the preparation can be done by the teacher in the classroom activity. Hence, sufficient attention is required to design a classroom activity that gives effective results. A certain amount of computer literacy to the teacher is needed to use E-learning resources in classes. While designing activities, this factor supposed to be taken care.

4.1. Ten classroom Activities

The ten classroom activities that were developed as part of the Study work are designed by giving due consideration to all the design aspects that were discussed earlier and they also are aimed at reflecting all the key aspects of CLL.

The ten classroom activities are focused on developing listening skills to meet everyday listening tasks. They are designed for students who belong to the first year of Graduate studies. The activities are designed to accommodate forty students. The ten classroom activities are based on three basic classroom activities. The first four activities follow the 'Model 1' design, the next three activities follow the 'Model 2' design and the last three activities follow the 'Model 3' design. Blackboard and chock, books and work sheets, and computers with internet access and multimedia capability, and mobile phones with headsets are to be utilized in conducting the classroom activities. Each activity is of forty-five minutes in duration as the average class period is about one hour. Fifteen minutes of the class period was allotted for classroom management and other routine work such as handling attendance. The three basic design models are used in designing the ten activities and their highlights. This helps in throwing light on various design aspects that are unique to activities that are based on CLL.

The planning of the four activities has taken into account all the limitations and realities of their classroom. The average class period is about sixty minutes and therefore each classroom activity is designed to be completed in forty-five minutes or there about after having considered a time leave of fifteen minutes for routine classroom management tasks such as maintaining the attendance etc…

Each activity is divided into three stages viz. preparation, instruction, and procedure. The time allotment for the preparation is not included in the duration of the activity as fifteen minutes of the class is already allocated for classroom routines and arrangement. The instructional phase is given three minutes of the activity time in which the teacher is expected give the instructions for performing the activity. The instructions should be clear and if needed shall be given in the first language.

The procedure of the activity is divided into ten steps which are initiation, encouragement, translation and correction, recording, monitoring,

transcription, discussion, highlighting, confession & reflection, and conclusion.

4.1.1. Model 1

'Model 1' design is suitable for the initial part of the course. It divides the class into large groups yet all the groups work towards a common goal of listening to an audio clipping. Large groups allow a certain amount of comfort level to students to accommodate to the new environment. The purpose of the four activities based on the 'Model 1' design is very basic. These activities are designed to initiate the students to overcome their difficulties in listening to English.

4.1.2. Model 2

The next batch of activities 5, 6, and 7 are based on 'Model 2' design the purpose of the first four activities are to bring the students out of their inhibition towards listening in English, the purpose of these three activities is to indulge them in simple questioning and answering. As questioning and answering forms the basic structure of any conversation, the aims at helping students acquire these language aspects. Keeping the CLL spirit, topics of discussions of these activities will be determined by the students when they participate in the activities. Though the purpose of activities is to develop questioning and answering skills, the activities will be taken by the students in groups.

The three activities based on 'Model 2' design, The focus of the activities is to make students listen more. The class as a whole discusses the topic of the listening exercise. The teacher follows this discussion with highlighting of key language elements.

4.1.3. Model 3

The 'Model 3' design divides the class into the smallest groups i.e. pairs of students in order to make every student attempt to listen to English. However, the students are not isolated. Four groups of two, i.e. eight students still sit together in a pool and each pair has an opportunity to listen to an audio sample and develop their listening skills.

The topic of the classroom activity would be long speeches and therefore, the question setter will ask five questions to the student who is playing the role of the answer giver. The answer giver will answer the five questions. Students will either ask questions or give answers. They all will utter the sentences and record them. However, the entire activity will take place with the background of congeniality and no demand.

4.2. Advantages of Community language learning

This design encourages the individual student to contribute more to the group work, this design creates a very similar environment to that of the 'Model 1' design.

Therefore, students feel the same secured and friendly atmosphere in 'Model 2' design as well. These activities are not much different when it comes to the level of proficiency expected from the students. These three activities are the logical progression from the first four activities. The performance expected is of basic listening skills with the ability to frame questions and answers. Therefore the three activities do not put much demand on the students in terms of their proficiency. However, as mentioned earlier, these students possess all the knowledge and competence required to indulge in casual conversations. Hence, the effort is focused on breaking their inhibitions of listening and speaking in English.

The three activities based on 'Model 2' design provides a practice ground for students to practice what they have learnt in the previous four activities, these three activities will also become different in content and language use. The three activities will also focus on some of the favourite topics of students like cinema and memes.

The last three activities also focus on helping students to acquire basic listening skills that would help them in participating in conversations. The purpose of these activities is to involve groups of students into more intense listening sessions comprising questions and answers based on audio clippings played in mobile devices and listened by individual students. These three activities are logical progressions of the previous seven activities. These activities also provide the same secure anxiety free environment to the students in order to interact with fellow students as a community and indulge in the learning process of English.

The purpose of the three activities based on the 'Model 3' design is to involve students in longer listening practices as well as in interactions. However, the students are expected to engage in the basic form of conversation. These activities reflect one of the key features of CLL – learning in an anxiety free, secure and motivating environment.

The study undertaken is considered to be significant in terms of the contribution it might make to the field of inclusive English Language

Teaching. By exploring and discussing the ways to design a classroom activity based on e-learning platform. ESL students can learn listening skills to listen and watch audio and video files effectively would go a long way in helping those students to have a command over English as used in the computers and mobile access information on the internet

5. E-LEARNING MATERIALS

The materials used in completing these activities will carry special significance as they are E-learning materials. All the advantages of using such materials can be utilized in delivering the activities. Features such as flexibility in content, improvisation of content as required by CLL methodology, motivational environment, etc. can be incorporated to make the activities effective. E-learning resources from six sources namely, Randal, ELLLO, English Central, TED Talks, YouTube, and English Listening App can be used in the activities.

The teacher can use his/her fullest discretion in deciding the type of material to be used that is available from these sources. Or, the teacher can even allow students to choose the materials on the spot if they have technical resources to provide faster internet connectivity that can provide instant playing of audio clippings. The classroom activities can be extended further with the course of the students' discussion supported by additional materials that can be searched and accessed over the internet. E-learning materials offer the flexibility, that CLL activities require to not only capture the students' attention, but also to provide a friendly atmosphere for pressure-free learning.

The resources available are updated frequently and therefore the content would be current in nature. This can further allow the students to update themselves in terms of current affairs. CLL activities are primarily student-centered and student-driven.(5) Therefore, it would be improper to confine the designs to a particular type of content. This is the reason why there are no specific resource mentioned here. Perhaps an implementation of the classroom activities can provide such details, which is beyond the scope of the present study.

Appendix A

Classroom Activity Model 1

Name of the Activity : Listening Activity [n]

General Objective : Developing listening Skills

Methodology Adopted : Community Language Learning

Duration of the Activity : 45 min

No. of participants : 40

Class structure : 8 groups of 5 members each

Teaching aids & materials : Computers with internet access and multimedia capability, blackboard an chalk, paper and pen

Other skills focused : Writing and Speaking

Preparation [5 minutes before the activity begins]

The class is to be divided into 8 groups of 5 members each through any selection method such as lot system, calling numbers and grouping multiples of numbers etc. if preferred, the teacher can have mixed groups with boys and girls present in each group or s/he can make a all boys and all girls groups. Each group is to sit in a semicircle with members facing the computer, and should be able to observe the blackboard. Each student can have paper and pen at his/her disposal. The teacher is to have access to the blackboard, computers, and all groups.

Instructions

(to be given to the students at the beginning of the activity and it may be in first language, Tamil)

We are going to listen to an audio clipping from the internet [from any of the five web sites]. You are to listen carefully. Each group has to come up with one questions related to the content of the video. The group can discuss among themselves what question to ask. Once you decide, you have to call me and give me the question in either English or Tamil. I will translate the question or correct it to make it a proper sentence in English. one of the group members should write the question on the board. Once each group comes up with their question, we will listen to the entire audio clipping once again. Each group then will write the answers on a paper. Answers can then be discussed with other groups. Additional information on the content of the audio clipping can be exchanged. You can come up with your ideas in Tamil and I will help you translate them into English.

Step 1 – Initiation

The teacher can ask leading questions related to the content of the clipping.

Step 2 – encouraging & supporting

Students who are willing to come up with questions can be supported or encouraged by the group applauding him/her.

Step 3 – Translation

Teacher shall translate or correct the sentence that a group has come up with in an encouraging tone. (fault-finding tone or that which expresses disappointment should be avoided)

Step 4– recording

Each sentence is to be recorded by one of the students in each group on the blackboard.

Step 5 - – monitoring

The teacher should monitor what's going on in each group and pay extra attention to the groups that do not contribute to the activity.

Step 6 – Transcription

The teacher should observe the board as the students write their questions, and rectify the errors if any.

Step 7 – discussion

The teacher should encourage each group to talk about the audio clipping among themselves by reading the questions.

Step 8 – highlighting

The teacher should help students take notice of important language aspects such as word order or a particular expression in order to help them retain for future use.

Step 9 – confession and reflection

The teacher should encourage students to come up with their own ideas individualy and also allow them to express their experience and feedback on the listening activity. The teacher should not corner single students and pressurized them.

Step 10 – conclusion

The teacher should conclude the activity by appreciating students' participation in an encouraging tone and should create a secured and congenial atmosphere in the class for future activities.

Time allotment

Instructions - 3 min

Step 1, 2, & 3 - 8 min

Steps 4 & 5 - 8 min

Steps 6 & 7 - 8 min

Step 8 - 8 min

Step 9 - 8 min

Step 10 - 2 min

Appendix B

Classroom Activity Model 2

Name of the Activity : Listening Activity [n]

General Objective : Improving Listening Skills

Methodology Adopted : Community Language Learning

Duration of the Activity : 45 min

No. of participants : 40

Class structure : 10 groups of 4 members in each group

Teaching aids & materials : Computer with internet access and multimedia capability, blackboard and chalk, paper and pen

Other Language Skills : Speaking, and Writing

Preparation [five minutes]

The class is to be divided into 10 groups of 4 members each through any selection method such as lot system, calling numbers and grouping multiples of numbers etc. if preferred, the teacher can have mixed groups with boys and girls present in each group or s/he can make a all boys and all girls groups. Two groups are to sit – one group facing the other and should have visual access to the computer screen, teacher, and other groups. Each student can have paper and pen at his/her disposal. The teacher is to have access to the computer, and all groups.

Instructions

(to be given to the students at the beginning of the activity and it may be in first language, Tamil)

We are going to listen to an audio clipping from the internet [from any one of the five web sites]. Of the two groups that are facing each other, each group will come up with a question and the other group has to come up with an answer for the question. The group can discuss among themselves what to say or ask about the clipping. Once all the pairs of groups complete their questions and answers, they should present the questions to the entire class and allow them to answer the question. The question session can be followed by discussions on the content of the audio clipping.

Step 1 – Initiation

The teacher can ask leading questions related to the content of the clipping.

Step 2 – encouraging & supporting

Students who are willing to come up with questions can be supported or encouraged by the group applauding him/her.

Step 3 – Translation

Teacher shall translate or correct the sentence that a group has come up with in an encouraging tone. (fault-finding tone or that which expresses disappointment should be avoided)

Step 4– recording

Each question and answer is to be recorded by one of the students in each group on a paper.

Step 5 - – monitoring

The teacher should monitor what's going on in each group and pay extra attention to the groups that do not contribute to the activity.

Step 6 – Transcription

The teacher should observe the papers as the students write their questions, and rectify the errors if any.

Step 7 – discussion

The teacher should encourage each group to talk about the audio clipping among themselves by reading the questions.

Step 8 – highlighting

The teacher should help students take notice of important language aspects such as word order or a particular expression in order to help them retain for future use.

Step 9 – confession and reflection

The teacher should encourage students to come up with their own ideas individualy and also allow them to express their experience and feedback on the listening activity. The teacher should not corner single students and pressurized them.

Step 10 – conclusion

The teacher should conclude the activity by appreciating students' participation in an encouraging tone and should create a secured and congenial atmosphere in the class for future activities.

Time allotment

Instructions - 3 min

Step 1, 2, & 3 - 8 min

Steps 4 & 5 - 8 min

Steps 6 & 7 - 8 min

Step 8 - 8 min

Step 9 - 8 min

Step 10 - 2 min

Appendix C

Classroom Activity Model 3

Name of the Activity : Listening Activity [n]

General Objective : improving listening Skills

Methodology Adopted : Community Language Learning

Duration of the Activity : 45 min

No. of participants : 40

Class structure : 20 two-member groups

Teaching aids & materials : Mobile phones with internet access and multimedia capability, paper and pen

Other skills focused : Speaking and writing

Preparation

The class is to be divided into 20 groups of 2 members each through any selection method such as lot system, calling numbers and grouping multiples of numbers etc. if preferred, the teacher can have mixed groups with boys and girls present in each group or s/he can make a all boys and all girls groups. Each pair is to sit facing each other. One pair should have the access of one mobile phone with a headset. The students can have access to paper and pen.

Instructions

(to be given to the students at the beginning of the activity and it may be in first language, Tamil)

We are going to have a listening activity [Audio clipping from one of the five web sites is to be played]. Each pair will have to listen to one audio clipping. One student in the pair should listen to the audio and prepare five questions and hand the question paper to the other student. The second students should then listen to the audio and answer the five questions. The pair then should discuss the question and answers. They can repeat the activity

by changing the roles: the student who answered the questions earlier can become the question setter. They then should report to the teacher about their listening activity.

Step 1 – Initiation

The teacher can help the pairs decide who is to perform which role if the members are unable to decide.

Step 2 – encouraging & supporting

The teacher can always have encouraging words for students who lack in confidence or who hesitate to open up.

Step 3 – Translation

Teacher shall translate or correct the sentence that a pair has come up with in an encouraging tone. (fault-finding tone or that which expresses disappointment should be avoided)

Step 4– recording

Each listening session should have five questions and answers which are to be written on a paper.

Step 5 – monitoring

The teacher should monitor what's going on in each group and pay extra attention to the groups that do not develop their questions and answers.

Step 6 – Transcription

The teacher should check the transcriptions of the listening sessions.

Step 7 – discussion & Presentation

The teacher should allow the pairs to discuss the content of the audio clipping.

Step 8 – highlighting

The teacher should help students take notice of important language aspects such as word order or a particular expression in order to help them retain for future use.

Step 9 – confession and reflection

The teacher should encourage students to come up with their own ideas individuallly and also allow them to express their experience and feedback on the conversation. The teacher should not corner single students and pressurise them.

Step 10 – conclusion

The teacher should conclude the activity by appreciating students' participation in an encouraging tone and should create a secured and congenial atmosphere in the class for future activities.

Time allotment

Instructions	- 3 min
Step 1, 2, & 3	- 8 min
Steps 4 & 5	- 8 min
Steps 6 & 7	- 8 min
Step 8	- 8 min
Step 9	- 8 min
Step 10	- 2 min.

6. CONCLUSION

The purpose of using E-learning materials in CLL-based activities is that it can preserve the flexibility required for the successful execution of these activities. The primary objective of CLL is to create a pressure-free environment where students enjoy the learning process. Using E-learning materials, they offer flexibility, can deliver learning activities that are in vogue with CLL methodology and its spirit.

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RESEARCH ARTICLE

MULTICULTURALISM AND ASPECTS OF GLOBALISATION IN KIRAN DESAI'S *INHERITANCE OF LOSS*

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ABSTRACT

Indian English Literature pertains to the body of work by writers from India, who pen strictly in the English language and whose native or co-native language could be one of the numerous regional and indigenous language of India. English literature in India is also intimately linked with the works of associates of the Indian Diaspora. Among other writers, Kiran Desai is one of the most renowned writers in the Indian English Literature. With Kiran Desai, a literary tradition is reborn. One of the major themes in the novel is multiculturalism. Multiculturalism relates to communities containing multiple cultures. The term is used in two broad ways, either descriptively or normatively. As a descriptive term it usually refers to the simple fact of cultural diversity. It is generally applied to the demographic make-up of a specific place, sometimes at the organizational level, eg: school, businesses, cities, or nations. As a normative term, it refers to ideologies or policies that promote this diversity or its institutionalization. In this sense, multiculturalism is a society at ease with the rich tapestry of human life and the desire amongst people to express their own identity in the manner they see as fit. Such ideologies or policies vary widely, including country to country. Another major theme in the novel is globalization, which is a process of international integration arising from the interchange of world views, products, and other aspects of culture. Advances in transportation and telecommunications infrastructure, including the rise of the telegraph and its posterity the Internet, are major factors in globalization, generating further interdependence of economic and cultural activities. The term globalization has been increasing use since the mid-1980s and especially since the mid-1990s. The term *globalization* is derived from the word *globalize,* which refers to the emergence of an international network of social and economic systems. This paper attempts to analyze Kiran Desai's novel *The Inheritance of Loss* to bring out the various aspects of multicultural clashes and globalization.

Keywords: Diaspora, Globalisation, Multiculturalism, Post-Colonialism.

1. INTRODUCTION

Indian English Literature is an open stage to depict the profitable pearls of Indian Writing in English. From being a particular and excellent, India saw new type of Indian culture and voice is advancing and turning out as a result of works in English in which India banters consistently. Since pre - Independence time, Indian Writers - artists, authors, writers, and dramatists and so forth have been making earth shattering and significant commitments to world writing, the previous couple of years have seen a sudden development of Indian English Writing in the worldwide market. Indian English writing bear on to the made excellence of the scholars of India, who writes in English dialect and whose local dialect could be one of the various territorial dialect of India. Indian English writing is likewise profoundly interconnected with crafted by partners of the Indian diaspora, in particular with individuals like Kiran Desai, Salman Rushdie, who were born in India yet by and presently live somewhere else.

Indian English Literature has accomplished an autonomous status on the planet Literature. Wide scopes of subjects are managed inside Indian Writing in English. India's significant commitment to world writing is because of the inventive abstract works produced by Indian scholars also, authors in English. Their works mulled over and thought on different scope of issues like patriotism, flexibility battle, social authenticity, singular cognizance and so on. English has gained an uncommon benefit and fame in India particularly among the upper and the working classes. It is progressively being utilized by essayists to offer shape to the clashing difficulties and issues that defy the human mind.

2. MULTICULTURALISM IN *THE INHERITANCE OF LOSS*

Kiran Desai's *Inheritance of Loss* mainly underlines multiculturalism. Multiculturalism has been legitimate arrangement in a few western countries. Since 1970s, for reasons that differed from nation to nation, including the way that a large number of the colossal urban areas of the western world are progressively made of a mosaic of societies. Another significant topic in the novel is globalization, which is a procedure of universal mix emerging from the exchange of world perspectives, items, and different parts of culture.

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The Inheritance of Loss is set in Kalimpong, a peninsular augmentation of India into the border of Nepal, Tibet and Bhutan. Kiran Desai's second book concerns about what she calls the enormous anxiety of being foreigner. The novel makes Kiran Desai as a standout amongst the most discerning authors. Her novel moves between a few universes at a practically amazing velocity from the mist filled universe of Kalimpong to the dingy basement of New York, where she portrays with sincerity and sympathy the hidden universe of stow ways and illegal work. It incorporates a few periods too, while being set in the 1980s; it likewise recounts an account of life under the shadow of colonial rule. It is additionally about how individuals live in a similar milieu, bound by great relations and sick feeling; the book talks about alienation and fellowship at the same time. She enlightens the pain of outcast and the ambiguities of post-colonialism with a woven artwork of bright characters: a disillusioned old judge; Sai, his sixteen-year orphaned granddaughter; a talkative cook; and cook's child Biju. The book straightforwardly says the life and individuals of various conditions of India and in addition the multicultural conflicts between various nations.

The first multicultural clash in Desai's novel happens in 1986, in Kalimpong, high in the northern Himalayas. In the beginning of the story, it is rumored that the insurrection in the hills changed into resistance movement stockpiling men and guns. “It was the Indian-Nepalese this time, fed up with being treated like the minority in a place where they were the majority. They wanted their own country, or at least their own state, in which to manage their own affairs. Here, where India blurred into Bhutan and Sikkim… it had been always a messy map”(1).

The fundamental characters living here are the judge, the cook and Sai. Desai presents her characters as they experience their troublesome lives out of sight of proceeding with battles. One can see that Desai depicts the mortification and powerlessness of their characters against forceful conduct. They are automatically associated with the battles over the illustration outskirts. The conflict shows up between the two societies of Hindus and Nepalese and it unmistakably announces the way that violence and injustice play are skillfully disguised under misrepresentations of liberty and public interests.

Multicultural clash which develops through the depiction of Biju's life, partitioned into discrete circumstance. Biju is an Indian based American. He cleared out India with a fantasy of having better life in America. For the second time, he applies for a tourist visa and this time he is really successful and he gets it. One of the applicants, also asking for a visa,says Biju: “you are the luckiest boy in the whole world…”(1). One can see from this situation that no matter what sort of job it is, no matter what money they have to pay, no matter if they are cheated, their expectations of better life in the United States are so big that they are willing to do anything.

From the historic perspective, Indians moved to the United States from different reasons. A considerable lot of them cleared out to think about in colleges; many were disappointed with political circumstance in India or searching for better openings for work as there were significantly higher livelihoods in the United States. Another huge factor poverty; the migrants looked to help their families from abroad.

After arriving at New York Biju‟s life faces a series of challenges. He just enters the alienated world as a migrant, as an exile. “Above, the restaurant was French, but below in the kitchen it was Mexican and Indian.” (1)

In this circumstance, one can see that Desai brings up the contempt amongst Indian and Muslims. These two don't much try to become acquainted with each other. Rather they rather receive prejudice and mentalities of their fathers. This demonstrates characters are capable neither basically think without anyone else assessment.

Another point of this multicultural clash is to demonstrate a place of ladies in India. Their part changed quickly midst of history but it remained as one of the important in the society. This conflict demonstrates Desai's expectation to portray the remorseless reality of ladies in India. Her obligation is to act as per the profoundly established conventions. Another extensively talked about issue in a few multicultural clashes is religion. The author deliberately portrays the showdown with various religious gatherings to bring up the genuine reasons out of sight. By means of the characters underline the way that as opposed to attempting to coordinate with each other, individuals manages viewpoints like whose God is better, who my kids should hate, and who need to battle with. Such religious holds keeps down an improvement of the nation and besides it makes pressure among individuals which at last outcomes in brutality and

murdering. This is additionally firmly associated with regional battles.

Another regular element in the novel is an effort of the characters to carry on in English way. In any case, the character judge obviously exhibits the outcomes of such behavior. They stay caught in their psyches and as time passes by they discover that they totally lose their feeling of personality. The main route how to exist is to imagine that they are another person. Be that as it may, they can't escape from themselves. The part of ladies is the main viewpoint the author depicts in an unexpected way. Kiran Desai is normally significantly more interested by this issue.

The characters in Desai's book experience the ill effects of absence of certainty. They are totally resigned with destiny and don't invest sufficient effort to change their hopeless circumstance. The characters do not think from their own perspective and joins the majority with what they think and how they expect them to be. It likewise fills in as dreary rest of Biju's adventure to America and Jemu's trip to England are demonstration of that general concept. At last at that point Desai's portrayal of these immigrants significantly shows her feeling of the underside of globalization with in her novel.

3. THE VARIOUS ASPECTS OF GLOBALISATION

The creator communicates her misery on the way that parallels exist inside the limits of home culture additionally, giving a larger number of offices to foreigners than to Indians it is the projection of the mediocrity soaked up through years of colonization and in addition inside the mind of transients of our own nation. She concedes that treating individuals from a rich nation well and individuals from poor nation seriously.

The Inheritance of Loss delineates in its many points of interest the tragedies of the Third World nations simply got freedom from colonialism. The fundamental topic of the novel additionally gives off an impression of being the impact of the European powers in India and how Indians are haunted by the Colonization strategies. These impacts have mistreated and corrupted India. Her fiction is set in the modern day India and the stories described to portray the crumple of the established order because of the political unrest. Desai tries to deliver the issues of poverty and clues that globalization isn't a simple answer for the issues of the caught individuals of the lower social stratus.

Kiran Desai's second novel *The Inheritance of Loss* handles the about all influential waiting impacts of colonialism on two classes of South Asian individuals the individuals who endeavor to leave India and the individuals who stay in India. In her account Desai deftly carries amongst First and Third universes enlightening the torment of outcast stand up to their impacting interest. The country battles itself with the ambiguities of Post Colonialism and the blinding desire for a superior life where one individual's riches imply another's destitution. Through the characters Kiran Desai muses about her conceptualized status of India in the present globalized world which has been compacted with the rebellion of movement.

Multiculturalism envelops a scope of perspectives about the ramifications of developing cultural diversity. It bears every cultural communities, or, all the more extensively, all individuals, a similar social opportunities for self-articulation, correspondence, status and achievement. Distinctly societies in this way should be ensured and reinforced, especially when they have a place with minority or defenseless groups. Multiculturalists trust that diversity is attractive and ought to be commended as it is of an incentive to society at large.

The *Inheritance of Loss* captures a wide area like difference in nations, the present situation and the past, ethnic differences, religious views and social environment etc. The novel, set in India, demonstrates the ace craftsmanship of Kiran Desai in depicting an extensive variety of characters. It straightforwardly says the life and individuals of various conditions of India and additionally of various nationalities. Sai's life is a case for unity in decent variety. *The Inheritance of Loss* presents individuals who have faith in various religions and displaying different food habits. Distinction in dialect likewise adds to the decent variety of the novel. Additional attraction of this novel is though this novel is set in English many Hindi words are additionally observed and which create added beauty to this novel. The characters portrayed by Desai read books which are deeply rooted in multiculturalism and are different in the treatment of topic. Jemubhai goes with Tophams Law of Property, ICP – Indian Criminal Procedure, Indian Penal Code and other evidence act and other similar interests. The geology likewise displays contrast.

Biju's development really advances around New York are another striking case of the disparities that exist amongst westerners and non-westerners nationals and settlers the individuals

who profits by globalization and the individuals who don't. Biju's activity of conveying General Tso's Chicken and Szechuan wings to city inhabitants has him. "On a bicycle with the delivery bag on his handle bars a tremulous figure between heaving buses regurgitating taxies"(1)

The Inheritance of Loss by Kiran Desai draws a picture regarding the contrast in various aspects of life. Be that as it may, the gray had pervaded inside. The novel as it does in Cho Oyu, diffusing the borders and all noted divisions. In the other words some kinds of unity, can be found even the novel carries out diversity in the novel. Character legislative issues reinforce the aggregate personality and regular encounters of individuals in a general public. It sees the person as inserted in a specific social, social, institutional or ideological setting. A pride in one's way of life gives individuals a feeling of chronicled and social rootedness. Also, conversely, a frail or broke feeling of personality leaves individuals feeling isolated and befuddled. Multiculturalists acknowledge that individuals can have numerous personalities and different loyalties. For example, they can have steadfastness to their nation of origin and furthermore to the nation of their settlement.

Desai affirms that ethnicity and racial preferences are a general marvel and globalization can't manage the cost of any huge consolation. Kiran Desai presents the mind-boggling feeling of humiliation experienced by ethnic gatherings that arrive at the worldwide town of America to secure a superior future. Through the unresponsiveness of the life of Biju, she displays the status of unlawful immigrants and the sentiment of alienation frequently experienced by expatriates. Kiran Desai acknowledges that the underestimated groups are two way sufferers since they think that its hard to look for spaces in the worldwide society and also re alienated from the focuses of their own cultural identity. Each of the characters in the novel has the effect of globalization even the neighbors as well.

Kiran Desai, a certain and a skilled author, is rich with points of interest and displaying a beautiful picture of multicultural conflicts and the different parts of globalization. Desai's books are loaded with intelligence and unpretentious parallels. It is both funny and intensely tragic, however for the most part optimistic. The extremely egalitarian ideas of multiculturalism neglect to remain constant practically speaking in light of the unbalanced connection between one cultural group and the others relationship which safeguards the dichotomies amongst centres and edges. India and America may have pushed the multiculturalist's areas of identity political issues with a utopian vision of regarding all the distinction yet Desai deconstruct and destabilize this thought by demonstrating the logical inconsistency between multicultural ideologies and practices. She likewise implies that globalization isn't a simple solution for the issues of the caught individuals of the lower social stratus.

4. CONCLUSION

Multiculturalism and globalization are the normal topics of post-colonial writers. These are taken as giant and ambiguous ideas that should have meaning. *The Inheritance of Loss* by Kiran Desai, a confident and a talented writer, is rich with details and presenting a picturesque of multicultural clashes and the various aspects of globalization. Desai’s novels are full of wisdom and subtle parallels. It is both funny and bitterly sad, but generally optimistic. Never preachy, never predictable, Desai’s scope is broad, looking at the consequences of large cultural and political forces for both people and individuals. She illustrates her themes without making moral judgments about her characters. Her writing is liquid and beautiful with delightful turns of phrases. The very egalitarian notions of multiculturalism fail to hold true in practice because of the lopsided relationship between one cultural group and the others relationship which preserves the dichotomies between centers and margins. India and America may have advocated the multiculturalist’s locations of identity politics with a utopian vision of respecting all the difference but Desai deconstruct and destabilize this idea by showing the contradiction between multicultural ideologies and practices. She also hints that globalization is not an easy solution to the problems of the trapped people of the lower social stratus. Pankaj Mishra says,

In fact, Desai's novel seems to argue that such multiculturalism, confined to the Western metropolis and academe, doesn't begin to address the causes of extremism and violence in the modern world. Nor, it suggests, can economic globalization become a route to prosperity for the downtrodden (2)

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RESEARCH ARTICLE

CHALLENGES FACED BY TAMIL LEARNERS IN LEARNING ENGLISH PRONUNCIATION

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ABSTRACT

Comparing the sounds of English and Tamil one can be able to acquire the knowledge of English sounds through his native. Only by studying the differences, difficulties of both English and Tamil it is possible to acquire a full understanding of the use of sounds. Phonological awareness refers to the ability to identify sounds of speech and make the necessary connection between the spoken units. And hence this paper attempts to discuss the differences between the languages and also the difficulties and the challenges faced by the Tamil learners in learning English pronunciation which helps the learners to identify the mistakes made by them and to be aware of the problems that forms the barrier. The possibilities will help the learners to be of a successful learner.

Keywords: Tamil leaners, English pronunciation.

1. INTRODUCTION

First language is the language through which the child makes acquaintance with everything to communicate. Learning of mother tongue takes place in very natural way of imitation and exposition. The language skills listening and speaking are learnt at home and then child is sent to school for learning other skills such as reading and writing of the language. The child has more exposure to use his mother tongue and possibility of getting corrected about the mistake that is made is more. Regional language is learnt by birth. But the process of second language learning is quite different from that of the first language learning. Learning of foreign tongue is an artificial process. Tamil learners as a second language learner of English, faces numerous challenges in the process of acquisition. In case of the status of Introduction of English Sound Pattern to Indian learner, there is no conscious effort to introduce the sound pattern and whatever knowledge a learner gathers is simply accidental.

2. RESEARCH QUESTIONS

1. What are the problems faced by the Tamil learners in learning English pronunciation?
2. Why is pronunciation important?

Following are some of the challenges faced by the Tamil learners.

3. CRITICAL PERIOD

Every individual acquires his/her native language as he grows. The language structure that he encounters first is his mother tongue and he gets familiar to its form and structure. By the time he attains the critical period he/she is well versed with his native language. As it is noted that language acquisition would be faster and efficient before the critical period, learners find it difficult to learn the second language and the language acquisition becomes a bit failure. The structures of L1 gets stick to the learner and later when introduced to the structures of l2, the clash occurs between features of L1 and L2.

4. LESS EXPOSURE

Tamil learners are not very much exposed to English language socially. When one gets well exposure to a language he is able to acquire the language automatically. A child is able to acquire the native language even if it is not taught to it. This is possible because the language exposure that exists. But in case of second language, Tamil learners lack in English exposure.

5. LACK OF PRACTICE

Tamil learners though they were made to learn English in schools, the present educational scenario does not provide essential knowledge. It doesn't fulfill the needs of the learners. No steps were taken in schools to make the students speak in English. They were only made to memorize things and just record things on examinations. The platform which should provide the space for the students to grow, fails to provide practice and thus without any practice of speaking the learner remain dumb in consideration with English.

6. INAPPROPRIATE TEACHERS

English is being taught right from the schools but even students could not make their

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pronunciation to be a successful one. This happens because school teachers aren't well versed so that they are not able to train their students. Our undeveloped curriculum syllabus doesn't provide necessary methodologies to improve one's speaking skills and so importance of pronunciation is hardly found. In most of the colleges phonetics sounds are handled in detail only at the level of master degree and so teachers who are qualified to the under graduate level in a way they aren't eligible to teach pronunciation as they don't have clear idea about that. So on one hand unqualified teachers create mispronunciations. And on the other hand the methodologies that is being followed in schools and colleges.

7. INFLUENCE OF L1 TAMIL

Most of the researchers agree that the learner's first language influences the pronunciation of the target language. This interference or influence from the first language causes errors in aspiration, stress and intonation in the target language.

English in Tamil Nadu is acquired as a second language through formal schooling. When a child learns a language through formal schooling, he learns it through the grids of his L1. When a child learns his L1 he does not learn sounds in isolation, but rather he learns the system. Having acquired the sound system of his L1 he tends to impose it on the sound system of the language he is learning. Tamilians learning English have been doing this for many years. When a child learns to speak a second language, the deeply ingrained patterns of his first language will interfere with those of the language he is learning. When a situation presents itself, the stronger associations of his first language will respond.

Native language plays an important role in the acquisition of second language. L1 features tend to affect L2 which creates a clash between the system of L1 and L2 in the minds of the learner. Due to this clash there occurs the transfer of features from L1 into L2. This type of transfer is called as interference. The learners transfer their L1 sound patterns into second language which results in mispronunciations.

Following are some of the interference or influence of Tamil over English.

In Tamil morphemes are generally made up of a consonant plus a vowel with no consonant cluster and it usually ends with a vowel. If there are consonants at the end, Tamil speakers add /u/ sound in the end. And so have problems in pronouncing words such as prompt. Eg. Book is pronounced as /buku/ instead of /buk/.

And also they drop the final /t/ in consonant clusters like /st/ /tʃt/ /-nst/ -skt/.

As Tamil lacks the sound /θ, z, ʒ/ Tamil speakers tend to replace these sounds.

/θ/ is replaced by /t/

/z / is replaced by /ʃ/ or /s/

/ʒ/ is replaced by /ʃ/ or /s/

Tamil learners use /e/ for English /ei/

Eg. Gate is pronounced as kate instead of /geit/

In Tamil the voiced plosives occur after vowel or after a corresponding nasal.

Eg. Country is pronounced as /coundry/

8. CONCLUSION

There are certain minimal challenges and difficulties are there in learning English pronunciation. But these difficulties can be overcome with certain practices. The main problem is that lack of awareness. The students are not aware of English. Thus the first and the foremost need is for the teacher to know the accurate sound pattern of English language and distinctly identify the subtle difference between that sound with its nearest sound in L1 of the learner. Thus by knowing the exact nature of both the sound systems in source language and the target language the teacher can progress in a way that she could convert the negative interference of L1 into positive influence in the teaching learning process. If they are taught by pointing out these differences and challenges they could be able to overcome. By comparing the sounds of English and Tamil he can be able to acquire the knowledge of English sounds through his native.

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RESEARCH ARTICLE

THE ADVANCEMENT OF ARTIFICIAL INTELLIGENCE A WAY TO MAN AND MACHINE IN COMBAT IN *TIME MACHINE* AND *I ROBOT*

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ABSTRACT

Science fiction has proved notoriously difficult to define. It can be explained as a combination of science and technology and development in robotics in short it can be otherwise called as 'realistic speculation about future events and a genre based on an imagined alternative to the reader's environment. It has been called a form of fantasy fiction and an historical literature. The paper goes further with two main concepts one with clash between two people of future and the other with advancement of science particularly on robotics. First is about general outline to science fiction in short a (SF) a genre cause problem because it does not recognize the hybrid nature of many SF works. It is more helpful to think of it as a mode or field where different genres and subgenres intersect. And then there is the issue of science. In the early decades of the 20th century, a number of writers attempted to tie this fiction to science and event to use it as a means of promoting scientific knowledge, a position which continues into what has become known as 'hard SF'. The research article is completely based on advancement of science and its effects.

Keywords: SF, AI and COBOT.

1. INTRODUCTION

Science-fiction (SF) is a period touchy subject in writing. Generally cutting edge, sci-fi hypothesizes about elective lifestyles made conceivable by mechanical change, and thus has now and again been designated "speculative fiction." Like dream, and regularly connected with it, sci-fi imagines elective universes with credibly predictable principles and structures, set apart by one way or another from the standard or well-known universe within recent memory and place. Unmistakable from dream, in any case, sci-fi thinks about innovation to think about how it may change the states of our reality and change being human. "Science fiction" (SF)is the class that thinks about what interesting new creatures we may progress toward becoming what mechanical structures we may concoct for our bodies, what systems and frameworks may support or tap our life energies, and what machine shells may contain our spirits. The following article is completely based on science fiction which particularly focuses on Robotics, Artificial intelligence, followed by its consequences arises

On investigating the term mechanical technology, which truly implies Robotics is a part of innovation which manages robots. Robots are programmable machines which are normally ready to do a progression of activities self-governing, or semi-self-ruling. As indicated by my feeling, there are three vital elements which comprise a robot:

1. Robots communicate with the physical world by means of sensors and actuators.
2. Robots are programmable.
3. Robots are normally independent or semi-autonomous (1).

As indicated by me robots are "normally" self-governing since a few robots aren't. Tele-robots, for instance, are totally constrained by a human administrator however tele-robotics is still classed as a part of apply autonomy. This is one model where the meaning of mechanical technology isn't clear.

It is shockingly hard to motivate specialists to concur precisely what establishes a "robot." Some individuals state that a robot must almost certainly "think" and decide. Nonetheless, there is no standard meaning of "robot thinking". Requiring a robot to "think" recommends that it has some dimension of computerized reasoning. Anyway you characterize a robot, apply autonomy includes planning, building and programming physical robots. Just a little piece of it includes man-made reasoning.

2. ARTIFICIAL INTELLIGENCE (AI)

Artificial intelligence (AI) is a part of software engineering. It includes creating PC projects to finish assignments which would some way or another requires human insight (2). Computer based intelligence calculations can handle learning, discernment, critical thinking, and

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dialect understanding as well as coherent thinking. Man-made intelligence is utilized from multiple points of view inside the advanced world. For instance, AI is widely used in Google seeks, Amazon's suggestion motor and SatNav course discoverers. Most AI programs are not used to control robots. When AI is used to control robots, the AI calculations are just piece of the bigger mechanical framework, which additionally incorporates sensors, actuators and non-AI programming.

Artificial intelligence includes some dimension of machine realizing; where a calculation is "prepared" to react to a specific contribution to a specific route by utilizing known sources of info and yields. The main aspect that separates AI from increasingly regular writing computer programs is "insight." Non-AI programs just complete a characterized grouping of guidelines. Artificial intelligence programs emulate some dimension of human insight.

3. BOTS WITH AI and NON AI

Artificial intelligence acts as a bridge between Robotics and AI. These are robots which are constrained by AI programs. Numerous robots are not misleadingly insightful up until as of late, all mechanical robots must be modified to complete a dreary arrangement of developments. As we have talked about, tedious developments don't require man-made brainpower. Non-intelligence robots are very restricted in their usefulness. Computer based intelligence calculations are regularly important to enable the robot to perform progressively complex undertakings.

A simple robot (cobot) is an ideal case of a non-intelligence robot. For instance, you can without much of a stretch program a cobot to get an item and place it somewhere else. The cobot will at that point proceed to pick and place questions in the very same route until you turn it off. This is a self-ruling capacity on the grounds that the robot does not require any human contribution after it has been customized. Notwithstanding, the errand does not require any knowledge.

4. DIFFERENCE BETWEEN ARTIFICIAL INTELLIGENCE AND ROBOTICS

The main difference between robotics and artificial intelligence is that robotics, it is completely a branch of engineering and it involves not only the programs that drive it but also the machine parts like its design and construction. It generally needs people from various streams of studies and skills are required to develop the complete concept of robotics. Similarly, artificial intelligence has its development from computer science in simple it is completely a development of simple program. In broader sense, robotics involves both hardware and software, whereas artificial intelligence is all about software and coding.

The main objective of robotics is to perform tasks physically and reach out to the place where human beings cannot physically. The word “physically” is important because the main aim of robotics is to replace human beings so that the algorithms understand the external situation better and make decision and act similar to that of humans. Robotics can be in two ways, it would be autonomous or semi-autonomous depends upon the programmed tool or by the controller, in some cases self-constructive bots are used they could think similar like human and make decision very fast such bots are completely autonomous.

The scope of Artificial intelligence is very broad, it has to be analysed from the fact that only few percentage of AI is used in machine and the rest is available in online applications and electronic devices. The possibilities are infinity it can be programmed in such a way that it starts to understand the input and think various possible ways before producing an output. In several other situations there are possibilities that a robot could think in a different way as it gets exposed to the external circumstances, the advancement in AI would ultimately result in adopting a program. The advanced robots would never fail when the external situation and scenarios change drastically, especially when it is not programmed to respond accordingly.

Robotics and AI are completely different they are not similar, it is true that AI is used in robotics to make the robot smart but it has to be restricted to a level it would be better to avoid self-constructive robots.

5. RISE OF ROBOTS AGAINST MANKIND

Robots and artificial intelligence have taken our life to the next level, they have become our sixth finger. Our lives would be paralysed without these two major inventions. In our day to day life, bots with artificial intelligence play a serious role. The tech we use today are completely made of those two major inventions for e.g. computers, cell phones, auto driving in cars and aeroplane, quality checking and defence. We use programmed robots to perform particular task

without any break in operation such are called simple robots. Similarly these robots reduce manual error and gives perfection on completing its assigned task. In some cases humans are replaced by bots with special intelligence and to reduce manual error in such cases human loses their employment, in this case it takes the place of human.

On examining deep into it, the growth of artificial intelligence has to be brought under the control of human; there is a great possibility of war in future either it would be a man vs. man or mankind against robots. This concept is been said by the two great novelist

1. Herbert George wells – The Time Machine (3)
2. Issac Asimov – I Robot (4)

According H.G Wells, the concept of science fiction is been used in a different way. The author travels through the time with his device and finds out that there would be great possibility of war between two clan of people, particularly in this novel he explains through two human like creatures named as Morlocks and Eloi.

The second novel, I Robot which explains about Artificial intelligence and how robots develop their intelligence with artificial intelligence. The author Issac Asimov has used this idea in his novel, he has also given an idea about self-programmable robots which would create danger to mankind in future. Through this we could get a clear picture about future with bots, and so the development of artificial intelligence has to be under the control of human.

6. CONCLUSION

At present, the idea has gone invalid as the growth of artificial intelligence has gone beyond the control limits; we have introduced bots in all the fields now it has become a part of our society. The advancement in science has taken our life to the next level. The topics which these two authors have taken have already occupied our life, so there would be a great possibility of war in future. At present we have a bot named Sophia developed by Hong-Kong, Hanson robotics this bot is specially made with artificial intelligence. This robot has a physical appearance of human which is popularly called as humanoid bots. May in future there is a possibility of war between men vs. men or it would be war between men vs. robots.

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RESEARCH ARTICLE

ON THE CONTROLLABILITY OF IMPULSIVE FUNCTIONAL INTEGRO-DIFFERENTIAL EQUATIONS IN BANACH SPACES

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ABSTRACT
In this paper, we established the some sufficient conditions for controllability of impulsive functional integrodifferential equations with nonlocal conditions by using the measure of noncompactness and Monch fixed point theorem.
Keywords: Controllability, impulsive functional integro-differential equations, Banach space.

1. INTRODUCTION

Impulsive differential equations are a class of important models which describes many evolution process that abruptly change their state at a certain moment,see the monographs of Bainov and Simonov [2], Lakshmikantham et al.[8] and have been studied extensively by many authors[3,4,10]. On the other hand, the concept of controllability is of great importance in mathematical control theory. Many authors have been studied the control of nonlinear systems with and without impulses; see for instance[5, 6, 7].

The starting point of this paper is the work in papers [5,9]. Especially, authors in [9] investigated the controllability results of mixed-type functional integro-differential evolution equations with nonlocal conditions

$$x'(t) = A(t)x(t) + f\left(t, x_t, \int_0^t h(t,s,x_s)\,ds, \int_0^b k(t,s,x_s)\,ds\right) + Bu(t), \quad (1.1)$$

$$t\epsilon J = [0,b],\ t \neq t_i, i = 1, \dots, s,$$

$$\Delta x|_{t=t_i} = I_i(x_{t_i}), i\ 1, \dots, s, \quad (1.2)$$

$$x_0 = \emptyset + g(x),\ t\epsilon[-r, 0], \quad (1.3)$$

by using Monch fixed point theorem. And in[5], authors studied the following controllability of impulsive differential systems with nonlocal conditions of the form

$x'(t) = A(t)x(t) + f(t, x(t)) + (Bu)(t)$ a.e on $[0,b]$ (1.4)

$$\Delta x(t_i) = x(t_i^+) - x(t_i^-) = I_i(x(t_i)), i = 1, .., s. \quad (1.5)$$

$$x(0) + M(x) = x_0 \quad (1.6)$$

Motivated by above mentioned works[5,9],the main work of this paper is to prove the controllability results of impulsive integro-differential systems with nonlocal conditions.

$$x'(t) = A(t)x(t) + f(t, x(t)) + \int_0^t h(t,s,x(s))\,ds + (Bu)(t) \quad (1.7)$$

$$\Delta x(t_i) = x(t_i^+) - x(t_i^-) = I_i(x(t_i)), i = 1, .., s. \quad (1.8)$$

$$x(0) + M(x) = x_0 \quad (1.9)$$

Where $A(t)$ is a family of linear operators which generates an evolution operator

$$U(t,s): \Delta = \{(t,s)\epsilon[0,b] \times [0,b]: 0 \leq s \leq t \leq b\} \to L(X),$$

here, X is a Banach space, $L(X)$ is the space of all bounded linear operators in X; $f: [0,b] \times X \to X$; $G: [0,b] \times X \to X$; $O < t_1 < \dots\dots\dots < t_s < t_{s+1} = b$; $I_i = X \to X, i = 1, \dots, s$are impulsive functions; $M: PC([0,b]); X \to X$; B is a bounded linear operators from a Banach space V to X and the control function$u(\cdot)$ is given in $L^2([0,b], V)$.

The paper is organized as follows: In section 2, we will recall some basic notations definition, hypothesis and necessary preliminaries. In section 3, we prove the controllability of impulsive integro-differential system with nonlocal system(1.7) –(1.9), using Monch fixed point theorem.

2. PRELIMINARIES

In this section, we recall some basic definitions and lemmas which will be used to prove our main results of this paper.

Let (X,‖.‖) be a real banach space .We denote by C([0,b];X) the space of X- valued continuous function on [0,b]with the norm $\|x\| = \sup\{\|x(t)\|, t \in [0,b]\}$ and by $L^1([0,b];X)$ the space of X- valued Bochner integrable functions on[0,b] with the norm $\|f\|_{L^1} = \int_0^b \|f(t)\|\,dtS$.

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For the sake of simplicity, we put J=[0,b] ; J_0 = $[0,t_1]$; $J_i = (t_i , t_{i+1}]$,i=1,.....,s. In order to define the mild solution of problem (1.7)-(1.9), we introduce the set PC([0,b];X) = {u : [0,b]→X : u is]; continuous on J_i ,i = 0,1,....,s and the right limit u(t_i^+) exists, i = 1.....,s}. It is easy to verify that PC([0,b];X) is a banach space with the norm $\|u\|_{PC}$ = sup{ $\|u(t)\|$,t ε [0,b]} .

Definition 2.1: Let E^+ be the positive cone of an order Banach space (E,≤) . A function Φ defined on the set of all bounded subsets of the Banach space X with values in E^+ is called a measure of noncompactness (MNC) on X if $\Phi(\overline{co}\Omega) = \Phi(\Omega)$ for all bounded subsets $\Omega \subset X$,where $\overline{co}\Omega$ stands for the closed convex hull of Ω. The MNC Φ is said:

(1)Monotone if for all bounded subsetsΩ_1, Ω_2 of X we have:

$(\Omega_1 \subseteq \Omega_2) \Rightarrow (\Phi(\Omega_1) \leq \Phi(\Omega_2))$;

(2)Nonsingular if $\Phi(\{a\} \cup \Omega) = \Phi(\Omega)$ for every a ϵX ,$\Omega \subset X$;

(3)Regular if $\Phi(\Omega) = 0$ if only if Ω is relatively compact in x.

One of the most important examples of MNC is the noncompactness measure Of Hausdorff β defined on each bounded subset Ω of X by $\beta(\Omega)$= inf { ε> 0;Ω can be covered by a finite number of balls of radii smaller than ε}.for all bounded subset Ω, Ω_1, Ω_2 of X,

(1) $\beta(\Omega_1 + \Omega_2) \leq \beta(\Omega_1) + \beta(\Omega_2)$, where $\Omega_1 + \Omega_2 = \{x+y : x \epsilon \Omega_1, y \epsilon \Omega_2\}$

(2) $\beta(\Omega_1 \cup \Omega_2) \leq \max\{\beta(\Omega_1) , \beta(\Omega_2)\}$;

(3) $\beta(\lambda\Omega) \leq |\lambda|\beta(\Omega)$ for any $\lambda \epsilon$ R;

(4) If the map Q : D(Q) ⊆ X →Z is Lipschitz continuous with constants k,then $\beta_Z(QZ) \leq k\beta(\Omega)$ for any bounded subset $\Omega \subset D(Q)$, where Z is a Banach space.

Definition 2.2: A two parameter family of bounded linear operators U(t, s), 0 ≤ s ≤ t ≤ b on X is called an evolution system if the following two conditions are satisfied:

(i) U(s, s) = I, U(t, r)U(r, s) = U(t, s) for 0 ≤ s ≤ r ≤ t ≤ b;

(ii) U(t, s) is strongly continuous for 0 ≤ s ≤ t ≤ b

and there exists M_1> 0 such that U(t, s) ≤ M_1 for any (t ,s) ϵ T.

Definition 2.3: A function x (·) ϵ PC([0,b];X) is a mild solution of(1.7)-(1.9) if

$$X(t) = U(t,0)[x_0 - M(x)] + \int_0^t U(t,s)\left[f(s,x(s)) + \int_0^s h(s,\tau,x(\tau))\,d\tau + Bu(s)\right]ds + \sum_{0<t_i<t} U(t,t_i)\,I_i(x(t_i))$$

,for all t ϵ [0,b], where $x(0) + M(x) = x_0$.

Definition 2.4: The system (1.7) –(1.9) is said to be controllable on the interval J if for every initial function $\varphi \epsilon$D and $x_1 \epsilon$ X, there exists a control u ϵL_2 (J, V) such that themild solution x(·) of (1.7) – (1.9) satisfies.$x(b) = x_1 + M(x)$.

Definition 2.5: A countableset $\{f_n\}_{n=1}^{+\infty} \subset L^1$([0,b];X) is said to be semicompact if:

(1) The sequence $\{f_n\}_{n=1}^{+\infty}$ is relatively compact in X for a.e. t ϵ [0,b]

(2) There is a function $\mu \epsilon L^1([0,b]; R^+)$ satisfying $sup_{n\geq 1}\|f_n(t)\| \leq \mu(t)$ for a.e.

tϵ [0,b].

Lemma 2.1: Let $\{f_n\}_{n=1}^{+\infty}$ be a sequence of function in L^1([0,b];R^+).Assume that there exist

$\mu, \eta \epsilon L^1([0,b];R^+)$ satisfying $\sup_{n\geq 1}\|f_n(t)\| \leq \mu(t)$ and $\beta(\{f_n(t)\}_{n=1}^{+\infty}) \leq \eta(t)$ a.e. t ϵ [0,b]. Then for all t ϵ [0,b], we have$\beta(\{\int_0^t U(t,s)f_n(s): n \geq 1\}) \leq 2M_1 \int_0^t \eta(s)\,ds$.

Lemma 2.2: Let (Gf)(t) = $\int_0^t U(t,s)\,f(s)ds$. If $\{f_n\}_{n=1}^{+\infty} \subset L^1$([0,b];X) is semicompact then the set $\{Gf_n\}_{n=1}^{+\infty}$ is relatively compact in C([0,b];X) and moreover , if $f_n \to f_0$, then for all t ϵ [0,b] ,

$(Gf_n)(t) \to (Gf_0(t)$as n→ +∞.

Lemma 2.3: Let D be a closed convex subset of a Banach space X and 0ϵD. Assume that F: D→X is a continuous map which satisfies Monch's condition, that is, M⊆ $Discountable, M \subseteq \overline{co}(\{0\} \cup F(M)) \Rightarrow \bar{M}$ is compact. Then, there exists xϵD with x = F(x).

3. CONTROLLABILITY RESULTS

We first give the following hypothesis:

(H1) A(t) is a family of linear operators , A(t): D(A) → x, D(A) not depending on t and dense subset of X, generating an equicontinuous evolution system {U(t,s) : (t,s) ∈ Δ}, i.e.,

(t,s) → $\{U(t,s)x: x \in B\}$ is equicontinuous for t > 0 and for all bounded subsets B.

(H2) The function f:[0,b]× $X \to X$ satisfies:

(i) For a.e. $t\epsilon[o,b]$,the function f(t,·):X →X is continuous and for all xϵX,the function $f(\cdot,x)$:[0,b] →X is measurable;

(ii) There exists a function mϵL^1([0,b];R^+) and a nondecreasing continuous function

Ω: $R^+ \to R^+$such that$\|f(t,x)\| \leq m(t)\Omega(\|x\|)$, x $\epsilon X, t\epsilon[0,b]$and

$$\lim_{n\to+\infty} inf\,\frac{\Omega(n)}{n} = 0.$$

(iii) There exists h$\epsilon L^1([0,b];R^+)$ such that , for any bounded subset D$\subset X$,

$$\beta\left(f(t,x(t))\right) \le h(t)\beta(x(t))\text{for a.e. } t\epsilon[0,b],$$

where β is the Hausdorff MNC

(H3) The function h:[0,b]$\times X \to X$ satisfies:

(i)For each t,$s\epsilon$[0,b],the function h(t,s,·):X →X is continuous and for all xϵX,the

function h(·,·,x):[0,b] →X is measurable;

(ii) There exists a function m$\epsilon L^1([0,b];R^+)$ such that

$\|h(t,s,x(s))\| \le m(t,s)\|x(s)\|$,
x$\epsilon X, t, s\epsilon[0,b]$and $\lim_{n\to+\infty} inf\frac{x(n)}{n} = 0$.

(iii) There exists $\zeta\epsilon L^1([0,b];R^+)$ such that , for any bounded subset D $\subset X$,

$$\beta\left(h(t,s,x(s))\right) \le \zeta(t,s)\beta(x(s))\text{for a.e } t\epsilon J,$$

For convenience let us take $L_0 = max\int_0^t m(t,s)ds$ and $\zeta^* = max\int_0^t \zeta(t,s)ds$

(H4)M : PC(J,X) → X is a continuous compact operator such that

$$\lim_{\|y\|_{PC}\to+\infty}\frac{\|M(y)\|}{\|y\|_{PC}} = 0;$$

(H5) The linear operator W:$L^2(J,V) \to X$ is defined byWu = $\int_0^b U(b,s)Bu(s)ds$ such that:

(i) W has an invertible operators W^{-1} which take values in $L^2(J,V)/kerW$ and there

exist positive constants M_2 , M_3 such that $\|B\| \le M_2$ and $\|W^{-1}\| \le M_3$;

(ii) there is $K_W \in L^1(J,R^+)$such that , for any bounded set Q$\subset X$

$$\beta\left((W^{-1}Q)(t)\right) \le K_W(t)\beta(Q)$$

(H6) Let $I_i: X \to X$, $isi = 1, \dots, s$ be a continuous operator such that:

(i) There are nondecreasing functions $I_i: R^+ \to R^+, i = 1, \dots, s$ such that

$\|I_i(x)\| \le I_i(\|X\|)$and $\lim_{n\to+\infty} inf\frac{I_i(n)}{n} = 0$, i=1,....,s.

(ii) There exist constants $K_i \ge 0$,such that$\beta\left(I_i(x(t)\right) \le K_i\beta(x(t))$. i=1,...,s.

(H7) The following estimation holds true:

L = $(M_1 + 2M_1^2M_2\|K_W\|_{L^1})\sum_{i=1}^s K_i + (4M_1 +$ *8M12M2KWL1hL1+ζ∗b<1*

Where $M_1 = sup\{\|U(t,s)\|, (t,s)\epsilon\Delta\}$

Theorem: Assume that (H1) – (H7) are satisfied,then the impulsive integrodifferential system

(1.7)-(1.9) is nonlocally controllable on J, provided that

$\frac{1}{n}[C_1 + C_2\|M(x_n)\| + C_3\Omega(n) + C_4\|x_n(\tau)\| +$
$C_5\sum_{i=1}^s I_i(n)] < 1$.

Proof : Using hypothesis (H5)(i),for every $x\epsilon PC(J,X)$,define the control

$$u_x(t) = W^{-1}\Bigg[x_1 - M(x_n) - U(b,0)[x_0 - M(x_n)] - \int_0^b U(b,s)\Bigg[f\left(s,x_n(s)\right) + \int_0^s h\left(s,\tau,x_n(\tau)\right)d\tau\Bigg]ds - \sum_{0<t_i<t} U(t,t_i)\,I_i\left(x_n(t_i)\right)\Bigg]$$

We shall show that, when using this control, the operator, defined by

$$(Gx)(t) = U(t,0)(x_0 - M(x)) + \int_0^t U(t,s)\Bigg[f\left(s,x(s)\right) + \int_0^s h(s,\tau,x(\tau))\,d\tau + Bu_x(s)\Bigg]ds + \sum_{0<t_i<t} U(t,t_i)\,I_i\left(x(t_i)\right) \quad (3.1)$$

has afixed point. This fixed point is then a solution of the system(1.7)-(1.9).Clearly

$x(b) = x_1 - M(x) = G(x)(b)$which implies that the system (1.7)-(1.9) is controllable.

We define G=$G_1 + G_2$ where

$(G_1x)(t) = U(t,0)(x_0 - M(x)) + \sum_{0<t_i<t} U(t,t_i)\,I_i(x(t_i))$

$(G_2x)(t) = \int_0^t U(t,s)\left[f\left(s,x(s)\right) + \int_0^s h(s,\tau,x(\tau))\,d\tau + Bux(s)ds\right.$

for all tϵ[0,b].subsequently,we will prove that Ghas a fixed point by using lemma2.3. (Monch fixed point theorem).

Step1: There exist a positive integer $n_0 \ge 1$ such that $G(B_{n_0}) \subseteq B_{n_0}$,where $B_{n_0} = \{x\epsilon PC(J,X): \|x\| \le n_0\}$.

Suppose the contrary. Then we can find $x_n\epsilon PC(J,X), y_n = Gx_n\epsilon PC(J,X)$,such that $\|x_n\|_{PC} \le n$ and$\|y_n\|_{PC} > n$ for every $n \ge 1$.

Now we have

$$y_n(t) = \mathrm{U}(\mathrm{t},0)(\mathrm{x}_0 - \mathrm{M}(x_n) + \int_0^t U(t,s)\Big[f\big(s,x_n(s)\big) + \int_0^s h(s,\tau,x_n(\tau))\,d\tau + Bu_{x_n}(s)\Big] + \sum_{0<t_i<t} U(t,t_i)\,I_i(x_n(t_i))$$

$$\|y_n\|_{PC} \le M_1(\|x_0\| + \|M(x_n)\|) + M_1\Omega(\|x_n\|_{PC})\|\mathrm{m}\|_{\mathrm{L}^1} + M_1 bL_0\|\mathrm{x_n}(\tau)\|_{PC}$$

$$\big\|u_{x_n}\big\|_{L^2} \le M_3[\|x_1\| + M_1\|x_0\| + (1+M_1)\|M(x_n)\| + M_1\Omega(\|x_n\|_{PC})\|m\|_{L^1} + M_1 bL_0\|\mathrm{x_n}(\tau)\|_{PC} + M_1\sum_{i=1}^{s} I_i\,(\|x_n\|_{PC}) \qquad (3.3)$$

Substituting (3.3) in (3.2) we get

$$< \frac{1}{n}\Big[C_1 + C_2\|M(x_n)\| + C_3\Omega(\mathrm{n}) + \mathrm{C}_4\|\mathrm{x_n}(\tau)\| + \mathrm{C}_5\sum_{\mathrm{i}=1}^{s}\mathrm{I_i(n)}\Big] \qquad (3.4)$$

where $C_1 = \Big[M_1 + M_1^2M_2b^{\frac{1}{2}}M_3\Big]\|x_0\| + M_1M_2b^{\frac{1}{2}}M_3\|x_1\|$

$C_2 = \Big[M_1 + M_1M_2b^{\frac{1}{2}}M_3 + M_1^2M_2b^{\frac{1}{2}}M_3\Big]$, $C_3 = \Big[M_1\|\mathrm{m}\|_{\mathrm{L}^1} + M_1M_2b^{\frac{1}{2}}M_3\|\mathrm{m}\|_{\mathrm{L}^1}\Big]$

$\mathrm{C}_4 = \Big[M_1bL_0 + M_1^2M_2b^{\frac{3}{2}}M_3L_0\Big]$, $\mathrm{C}_5 = \Big[M_1 + M_1^2M_2b^{\frac{1}{2}}M_3\Big]$

by passing to the limit as $n \to +\infty$ in (3.4),we get$1 \le 0$, which is a contradiction. Thus we deduce that there is $n_0 \ge 1$ such that $\mathrm{G}(B_{n_0}) \subseteq B_{n_0}$.

Step2: The operators $G$is continuous on$PC[0,b];X$ For thispurpose, we assume that

$x_n \to x$ in $PC[0,b];X$.Then by hypothesis (H4) and (H6), we have

$$\|G_1x_n \to G_1x\|_{PC} \le M_1\|M(x_n) - M(x)\| + M_1\sum_{i=1}^{s}\big\|I_i\big(x_n(t_i)\big) - I_i\big(x(t_i)\big)\big\| \qquad (3.5)$$

$$\|G_2x_n \to G_2x\|_C \le M_1\int_0^b \big\|f\big(s,x_n(s)\big) - f\big(s,x(s)\big)\big\|ds + M_1\int_0^b \Big\|\int_0^s [h\big(s,\tau,x_n(\tau)\big) - h(s,\tau,x(\tau))]d\tau\Big\|ds + M_1M_2b^{\frac{1}{2}}\big\|u_{x_n} - u_x\|_{L^2} \qquad (3.6)$$

$$\big\|u_{x_n} - u_x\big\|_{L^2} \le M_3[\|M(x_n) - M(x)\| + M_1\|M(x_n) - M(x)\| + M_1\int_0^b \big\|f\big(s,x_n(s)\big) - f\big(s,x(s)\big)\big\|ds + M_1\int_0^b \Big\|\int_0^s [h\big(s,\tau,x_n(\tau)\big) - h(s,\tau,x(\tau))]d\tau\Big\|ds + M_1\sum_{i=1}^{s}\big\|I_i\big(x_n(t_i)\big) - I_i\big(x(t_i)\big)\big\| \qquad (3.7)$$

By domination convergence theorem, we have

$\|Gx_n \to Gx\|_{PC} \le \|G_1x_n \to G_1x\|_{PC} + \|G_2x_n \to G_2x\|_C \to 0$, as n$\to +\infty$, ie., G is continuous.

Step 3: G(D) is equicontinuous on every J_i, i=1,...s. ie., D $\subseteq \overline{co}\big(\{0\}\cup G(D)\big)$ is also equicontinuous on every J_i.To this end, let $y\epsilon G(\mathrm{D})$ and $t_1, t_2\epsilon J_i$, $t_1 \le t_2$. There is x ϵ D such that

$$\|y(t_2) - y(t_1)\| \le \big\|[U(t_2,0) - U(t_1,0)]\big(x_0 - M(x)\big)\big\| + \int_0^{t_1}\big\|\big(U(t_2,s) - U(t_1,s)\big)\Big[f\big(s,x(s)\big) + \int_0^s h(s,\tau,x(\tau))\,d\tau + Bu_x(s)\Big]\big\|ds + \int_{t_1}^{t_2}\|\mathrm{U}(t_2,0)\|\Big\|\Big[f\big(s,x(s)\big) + \int_0^s h\big(s,\tau,x(\tau)\big)\,d\tau + Bu_x(s)\Big]\Big\|ds \qquad (3.8)$$

By the equicontinuity property of U(·, s) and the absolute continuity of the lebesgue integral, right hand side of the inequality equation(3.8) tends to zero independent of y as $t_2 \to t_1$.

Therefore G(D) is equicontinuous on every J_i

Step 4: Assume that D= $\{x_n\}_{n=1}^{+\infty}$. since G maps D into an equicontinuous family,G(D) is equicontinuous on J_i.Hence D $\subseteq \overline{co}\big(\{0\}\cup G(D)\big)$ is also equicontinuous on every J_i.

Now we shall show that (GD)(t) is relatively compact in X for each t$\in J$.

From the compactness of M(·) ,we have

$$\beta(\{(G_1x_n)(t)\}_{n=1}^{\infty}) \le M_1\sum_{i=1}^{s}K_i\beta\big(x(t_i)\big) \qquad (3.9)$$

for $t\epsilon[0,b]$ .by lemma(2.1),we have

$$\beta_V(\{u_{x_n}(s)\}_{n=1}^{\infty})$$

$$\leq K_W(s)\left[2M_1\int_0^b h(s)\beta\big(x(s)\big)ds + 2M_1\zeta^* b\,\beta\big(x(s)\big) + M_1\sum_{i=1}^{s} K_i\beta\big(x(t_i)\big)\right] \qquad (3.10)$$

Then this implies that

$$\beta(\{(G_2x_n)(t)\}_{n=1}^{\infty})$$

$$\leq 2M_1\int_0^b h(s)\beta\big(x(s)\big)ds + 4M_1^2M_2\left(\int_0^b K_W(s)ds\right)\left(\int_0^b h(s)\beta\big(x(s)\big)ds\right) + 2M_1\zeta^* b\,\beta\big(x(s)\big) + 4M_1^2M_2\left(\int_0^b K_W(s)\,ds\right)\zeta^* b\beta\big(x(s)\big) + 2M_1^2M_2\left(\int_0^b K_W(\eta)\,d\eta\right)\sum_{i=1}^{s} K_i\beta\big(x(t_i)\big) \qquad (3.11)$$

There fore

$$\beta((\mathrm{GD})(t))$$

$$\leq M_1\sum_{i=1}^{s} K_i\beta(x(t_i)) + \left(2M_1 + 4M_1^2M_2\left(\int_0^b K_W(s)\,ds\right)\right)\int_0^b h(s)\beta\big(x(s)\big)ds + \left(2M_1 + 4M_1^2M_2\left(\int_0^b K_W(s)\,ds\right)\right)\zeta^* b\beta\big(x(s)\big) + 2M_1^2M_2\left(\int_0^b K_W(\eta)\,d\eta\right)\sum_{i=1}^{s} K_i\beta\big(x(t_i)\big) \qquad (3.12)$$

we have

$$\beta(GD) = [(M_1 + 2M_1^2M_2\|K_W\|_{L^1})\textstyle\sum_{i=1}^{s} K_i + (4M_1 + 8M_1^2M_2\|K_W\|_{L^1})(\|h\|_{L^1} + \zeta^* b)]\beta\big(x(s)\big)$$

$$= L\beta\big(x(s)\big)$$

Where L is defined in (H7). Thus,from the Monch's condition, we get

$$\beta(D) \leq \beta(\overline{co}\big(\{0\}\cup G(D)\big) = \beta(G(D)) \leq L\beta(D)$$

Which implies that β(D) = 0, since hypothesis (H7) holds. So we have that D is relatively compact.Finally,due to lemma ,G has atleast a fixed point and thus the system (1.7)-(1.9) is nonlocally controllable on [0,b].

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RESEARCH ARTICLE

STRUCTURAL AND OPTICAL PROPERTIES OF CADMIUM SULFIDE NANOPARTICLES PREPARED BY PRECIPITATION METHOD

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ABSTRACT

Cadmium Sulfide nanoparticles were prepared by a simple and cost effective precipitation method. X-ray analysis revealed broad diffraction peaks indicating that the particles are of very small size. The prominent broad peaks at 2θ values of 26.48°, 43.90°, and 51.91°, which could be indexed to the (002), (110) and (112) direction of the hexagonal phase of CdS. Optical studies showed maximum absorbance in the UV region but minimum absorbance in the VIS-NIR regions make it an excellent material for screening off UV portion of electromagnetic spectrum in UV filters and sensors.

Keywords: Cadmium Sulfide, XRD, UV-Vis-IR.

1. INTRODUCTION

Semiconductor nanoparticles has been an interesting field of research for more than three decades because it gives an opportunity to understand the physical properties in low dimensions and to explore their potential applications in the field of optoelectronics [1-3]. The optical properties are particularly based on the large variations of the band gap as a function of particle size, which is a consequence of quantum confinement [4-6]. The confinement effect is observed for Cadmium Sulfide (CdS) nanoparticles when the particle sizes are equal to or less than 50 Å [4,5]. The optical properties of CdS nanoparticles have been extensively studied in recent years as this material exhibits pronounced quantum size effects [7]. A lot of work has been done on the synthesis of these nanoparticles, and a wet chemical synthesis has come up as a promising technique because of the ability to produce various sizes and large quantities of the nanoparticles [7,8]. Since very small nanoparticles have larger surface to volume ratios, many properties are directly related to the particle surface. The surface properties of the nanoparticles have been studied much less than the bulk properties, even though this information is of significant importance, and therefore many interesting aspects of nanoparticles are still not revealed. In the present work an attempt has been made to study the structural and optical properties of CdS nanoparticles prepared by chemical precipitating method.

2. EXPERIMENTAL

2.1. Synthesis of CdS nanoparticles

Aqueous solution of cadmium nitrate ($Cd(NO_3)_2.4H_2O$) was stirred for 1 hour at room temperature. Aqueous solution of sodium sulfide (Na_2S) was added drop wise to cadmium nitrate solution and was stirred for 2 hours. A precipitate with yellowish orange color was formed soon after the addition of Na_2S. The nanoparticles were initially purified by precipitating the particles with excess double distilled water and the solution obtained was centrifuged at 3000 rpm for 5 minutes. CdS nanoparticles were obtained after the precipitate was dried at 100^0C for three hours.

The structural properties were studied by X-ray diffraction (XRD) using Bruker AXS D8 Advance diffractometer with Cukα radiation (k = 1.5406 Å) operating at voltage of 40 kV and a current of 30 mA. Optical properties were studied by JASCO 670 UV–Vis–NIR spectrophotometer.

3. RESULTS AND DISCUSSION

The structural characterization of the CdS nanoparticles has been carried out by X-ray diffraction technique using CuKα radiation. Figure 1 shows the X-ray diffraction pattern of the prepared CdS nanoparticles. The X-ray diffraction peaks are found to be very broad indicating that the particles are of very small size. The x-ray diffraction pattern exhibits prominent broad peaks at 2θ values of 26.48°, 43.90°, and 51.91°, which could be indexed to the (002), (110) and (112) direction of the hexagonal phase of CdS.

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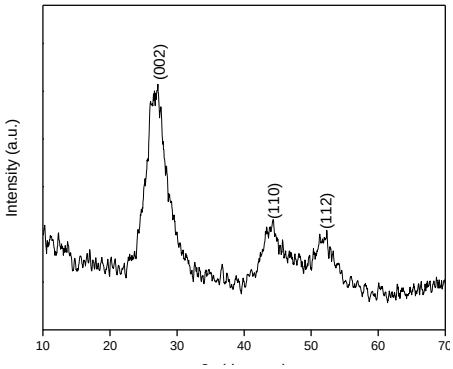

Fig.1. XRD spectra of CdS nanoparticles.

The lattice constants a and c have been determined from the interplanar spacing of the different (hkl) planes using the relation

$$(d_{hkl})^{-2} = \frac{4}{3}\frac{h^2 + hk + k^2}{a^2} + \left(\frac{l}{c}\right)^2$$

The evaluated lattice parameters of CdS are a = 4.142Å and c = 6.724Å, and are in good agreement with the standard JCPDS values (JCPDS# 02-0549). The average grain size has been determined using Scherer's equation.

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where, D is the grain size, K is a constant taken to be 0.94, λ is the wavelength of the x-ray radiation, β is the full width at half maximum and θ is the angle of diffraction. The crystallite size has been found to be 2.8 nm.

3.1. Optical properties

The optical absorbance spectrum of the CdS nanoparticles is shown in Fig. 2. The maximum absorbance occurred within the UV region from where the absorbance decreased with the wavelength towards the NIR region.

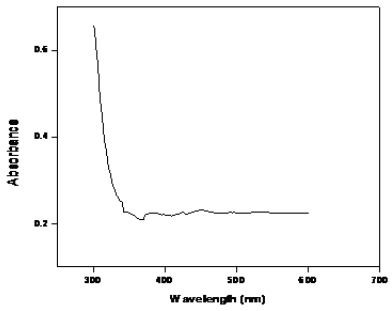

Fig. 2. Absorbance *vs* Wavelength plot

The properties of maximum absorbance in the UV region but minimum absorbance in the VIS-NIR regions make it an excellent material for screening off UV portion of electromagnetic spectrum in UV filters and sensors. The optical absorption in the shorter wave length region is mainly attributed to the electron transition from the top of the valence band to the bottom of the conduction band. The property of low absorptions (high transmittance) and low reflectance in the visible region makes the material a good candidate as transparent windows in solar cells.

4. CONCLUSION

A simple and cost effective precipitation method was used to prepare CdS nanoparticles. X-ray analysis revealed that the crystallite size of the CdS nanoparticles is found to be about 2.8 nm. Optical analysis showed maximum absorbance in the UV region indicated that the prepared CdS nanoparticles could be used as UV filters and Sensors.

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RESEARCH ARTICLE

ANTICANCER ACTIVITY OF SILVER NANOPARTICLES AGAINST HUMAN BREAST CANCER CELL LINE

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ABSTRACT

The present study demonstrated the effectiveness of bioinspired synthesized AgNPs against MCF-7 breast cancer cell line, we found a dramatic decrease in cell viability when the concentration of the bioinspired synthesized AgNPs was increased and there was a dose-dependent reduction in cell viability. This study further indicates the significance of green technology for nanoparticle fabrication and future application in control of several human diseases.

Keywords: Anticancer activity, silver nanoparticles, human breast cancer cell line.

1. INTRODUCTION

Cancer is considered as one of the most deadly disease in the world with high mortality. Subsequently, there are several cancer therapies available, chemotherapy has become an important component of cancer treatment for most cancers. In the area on oncology drug discovery, conventional chemotherapeutic agents still exhibit poor specificity in reaching tumor tissue and are often restricted by dose-limiting toxicity (1). Thecombination of developing controlled-release technology and targeted drug delivery many provides a more efficient and less harmful solution to overcome the limitations in conventional chemotherapy. Recent interest has been focused on developing nanoscale delivery vehicles, which are capable of controlling the release of chemotherapeutic agents directly inside cancer cell (2).

Nanomaterials are expected hopefully to modernise the cancer diagnosis and therapy. Nanoscale particles decorated with multiple functionalities are able to target and visualize tumor site via an imaging technology, thereby allowing of the early detection of cancer at begging stage onwards. Moreover, intelligent nanosystems can be constructed as controlled delivery vehicles which are capable of delivering anticancer drugs to a predetermined site and then releasing them with programmed rate, which can improve therapeutic efficancy (3). The advent of nanotechnology is considered to be the biggest engineering innovation since the industrial revolution. Proponents of the new technology promise to re-engineer the mam-made world, molecule by molecule sparking a wave of novel revolutionary biomedical products from machines to medicine (4). In inorganic nanoparticles, metal nanoparticles have received considerable attention in recent years because of their unique properties and potential applications in catalysis, photonics, optoelectronics, biological tagging and pharmaceutical application (1,5,6). The discovery and identification of new antitumor drug with low side on immune system has become an essential goal in many studies of immno-therapies (7).

The most effectively studied nanoparticles today are those made from Noble metals, in specifically Ag (8), Au (9) Pt and Palladium (10). The metal nanoparticles find vast applications in various fields ranging from medical to physical fields (6,11-13). Among these metallic nanopartilces, silver nanoparticles play a significant role in the field of biological system, living organisms and medicine (14). Nowadays the silver nanoparticles are one of the most commonly nanomaterials both in everyday life and in research laboratories. Silver nanoparticlesare incorporated into many commercial products including clothing/textiles, furniture, household appliances such as refrigerators, cosmetics and even children toys (15). Manikandan *et al*., (16) demonstrated that the biosynthesis of silver nanopartilces using ethanolic petals extract of *Rosa indica* exhibited potent antimicrobial property against human pathogenic bacteria, anticancer activity against human colon cancer cell and shows potent anti-inflammatory activity. This high degree of AgNPs commercialization has been achieved due to their significant antimicrobial and antifungal and

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anticancer activity (17). Hence, the aim of this present study deals with the *invitro* anticancer potential of silver nanoparticles (AgNPs) synthesized from the aqueous extract betel nut and betel leaf on MCF-7 human breast cancer cell line.

2. MATERIALS AND METHODS

2.1. Cell culture

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0C, 5% CO_2, 95% air and 100% relative humidity (18).

2.2. Synthesis and characterization of nanoparticles

Betel nut (BN) and betel leaf (BL) aqeoues extract mediated green synthesized silver nanoparticles preparation methodology, characterization and antibacterial properties were already reported.

2.3. In vitro cytotoxic of AgNPs

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/mL. About 100µl per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h100µl of medium containing the treated with various concentrations (25, 50, 100, 150 and 250 µg/ml) bioinspired synthesized AgNPs from betel nut and betel leaf aqueous extract. The treated cells were then incubated at 37˚C, 5% CO_2, 95% Air and 100% relative humidity for 48 h. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

2.4. MTT assay

After cell treatment process, the cells were then subjected for MTT assay. The stock concentration (5mg/ml) of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, a yellow tetrazole) in phosphate buffered saline (PBS) was prepared and 15µl of MTT was added in each AgNPs treated well and incubated at 37˚C for 4 h. The medium with MTT was then flicked off and the formed purple color formazan crystals were solubilized in 100µl of Dimethyl sulphoxide (DMSO), and read at 570 nm in a multi well ELISA plate reader. Each experiment was performed in triplicate for each experiment. *In vitro* Cytotoxicity was calculated at the percentage of viable cells at different concentration of sample relative to untreated (Control) cell. Optical density (OD) value was subjected to sort out percentage of cell inhibition by using the following formula (19).

$$\text{Percentage of viability} = \frac{\text{Mean OD value to experimental sample (AgNPs)}}{\text{Mean OD value to experimental control (Untreated)}} \text{ X } 100$$

Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population IC_{50}(Incubation Concentration) was determined.

2.5. Morphological observation

MCF-7 cells were grown and incubated with AgNPs at their IC_{50} concentration, the AgNPs treated and untreated plates were observed under amicroscope to detect morphological changes and photographed.

3. RESULTS AND DISCUSSION

Silver nanoparticles as a potent antimicrobial agent, is gaining greater demand in medical applications (14). At the same time, there are only limited studies in the cytotoxic effects of biological route synthesized silver nanoparticles, against human cancer cell lines. In this present experiment, MTT assay was used to assess the effects of AgNPs on proliferation of MCF-7 cells. Best of our knowledge, this is the first study to report the anticancer activity of silver AgNPs synthesized using aqueous extract betel nut and betel leaf against breast cancer cell lines (MCF-7). The obtained results of the present experiment revealed that the dose dependent cytotoxicity in AgNPs treated MCF-7 cells.

3.1. In vitro assessment of BN-AgNPs cytotoxicity

The aqueous extract of betel nut mediated bioinspired synthesized silver nanoparticles(BN-AgNPs) demonstrated a considerable cytotoxicity against MCF-7 human breast cancer cell lines at different concentration (25, 50, 100, 150 and 250 µg/ml). Figure 1 depict 87 % of cell death was observed in maximum concentration (250 µg/ml) of BN-AgNPs. More than 60 % of cell death was observed in concentration of 150µg/ml. Consequently, fifty percentage of cell death, which determines the inhibitory concentration (IC_{50}) value of bioinspired BN-AgNPs against human

breast cancer MCF-7 cells holds at around 108µg/ml.

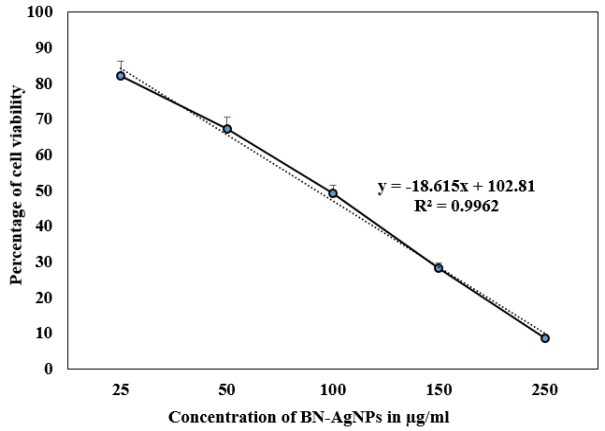

Fig. 1. Cytotoxicity effect of BN-AgNPs on cancer cell line MCF-7

Previously cytotoxicity effect of biological route synthesized silver nanoparticles has been reported against different cancer cell lines including, human lung cancer (A549) cell line (20), human epithelium cells (HEP G2) of liver cancer (21), human acute promyelocytic leukemia (HL-60) cell line (22) and human cervical carcinoma (Hela) cell line(23). Recently it was reported by Palaniappan*et al.* (24) that green synthesized colloids nano silver particles using *Cymodocea serrulata*leaf aqueous extract triggers cellular toxicity in treated potential against human lung cancer A549 cell lines. Hence, in this report we found that 150 µg/ml of bioinspired synthesized BN-AgNPs inhibits more than 60% of breast cancer cells.In addition, the microscopic observation of untreated and different concentration of BN-AgNPs treated cell lines showed in Figure 2. The improved cytotoxicity is mainly due to its easy permeability to the cellular barriers and its strong affinity towards biological macromolecules, addition it release reactive oxygen species that cause damages to cellular components via intercellular oxidative stress (25).

The aqueous extract of betel leaf mediated bioinspired synthesized silver nanoparticles(BL-AgNPs)revealed a significant cytotoxicity against human breast cancer MCF-7cell lines at different concentration (25-250 µg/ml). The obtain results of the present experiment indicated more than 90 % of cell death was observed in maximum concentration at 250 µg/mlof BN-AgNPs. At the BL-AgNPs concentration of 150µg/ml showed around 71 % of cell death. Thus, fifty percentage of cell death, which determines the inhibitory concentration (IC_{50}) value of bioinspired BL-AgNPs against human breast cancer MCF-7 cells holds at around 96 µg/ml. Cytotoxicity assays of BL-AgNPs achieved more than 50 % of cell death was observed in concentration of 100 µg/ml (Fig. 3).

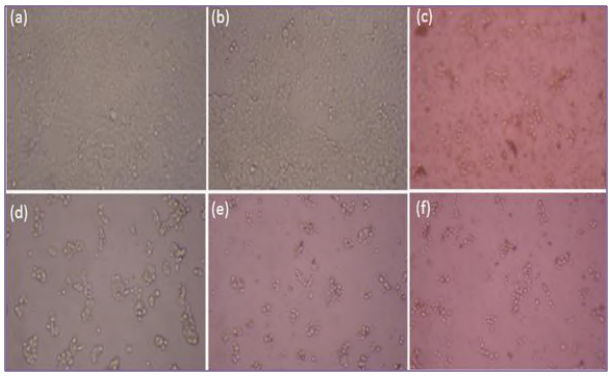

Fig. 2. Micscroscpic observation of MCF cell treated with BN-AgNPs (a) Control (Untreated) (b)25µg/ml, (c) 50 µg/ml, (d) 100 µg/ml, (e) 150 µg/ml and (f) 250 µg/ml. (More than 60 % of cell death observed at the concentration of 150 µg/ml).

3.2. In vitro assessment of BL-AgNPs cytotoxicity

Similar report of cytotoxicity was discussed by Sukirtha *et al*. (19) against Hela cell lines by using *Melia azedarach* mediated green synthesized AgNPs. Another report by Vivek *et al*. (18) noticeably discussed cytotoxicity activity of *Annona squamosa* extract reduced green synthesized AgNPs are exerting effect on human breast cancer MCF-7 cells in vitro at lower concentration level and did not affect the normal HBL (100) at lower concentration. Conversely, increased concentration of AgNPs produced significant toxicity against the normal HBL 100 cell. Similarly, our present experimental study revealed the dose dependent cytotoxicity was observed in AgNPs treated MCF-7 cells.

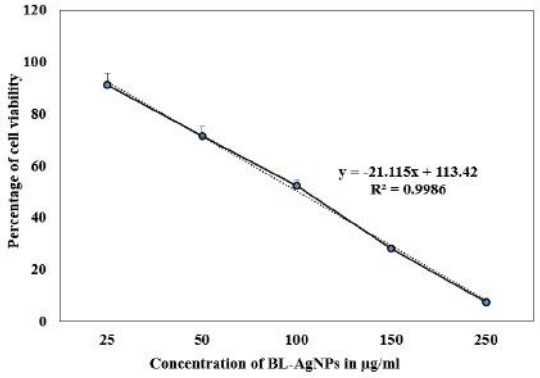

Fig. 3. Cytotoxicity effect of BL-AgNPs on cancer cell line MCF-7

A few in vitro studies have previously shown translocation of AgNPs in cancer cell with an IC_{50} value of 300µg/ml (26) 30 µg/ml (16). In fact, inside cancer cells AgNPs may induce reactive oxygen species and cause damage to cellar components leading to cell death (27). Likewise, our present study exposed the presence of 100 µg/ml of betel nut aqueous extract assets bioinspired silver nanoparticles (BL-AgNPs) is sufficient to inhibit the more than 50% of MCF-7 breast cancer cells (Fig. 4).

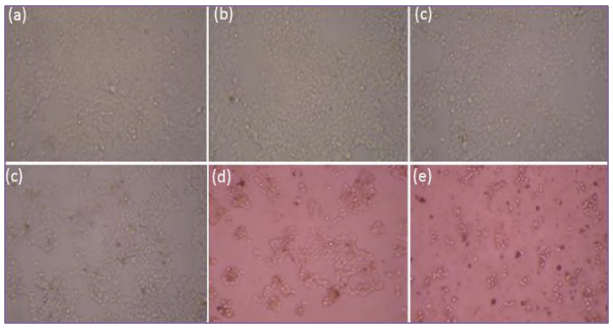

Fig. 4. Micscroscpic observation of MCF cell treated with BL-AgNPs (a) Control (Untreated) (b) 25 µg/ml, (c) 50 µg/ml, (d) 100 µg/ml, (e) 150 µg/ml and (f) 250 µg/ml. (More than 70 % of cell death observed at the concentration of 150 µg/ml).

4. CONCLUSION

The present study demonstrated the effectiveness of bioinspired synthesized AgNPs against MCF-7 breast cancer cell line, we found a dramatic decrease in cell viability when the concentration of the bioinspired synthesized AgNPs was increased and there was a dose-dependent reduction in cell viability. The decrease in cell viability with increase in AgNPs concentration, suggests that more number of AgNPs could accumulate inside cells resulting in enhanced stress, ultimately leading to cell death. The obtained results from the present study clearly show the enhanced effectiveness of the biologically synthesized AgNPs against MCF-7 breast cancer cells. This study further indicates the significance of green technology for nanoparticle fabrication and future application in control of several human diseases.

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RESEARCH ARTICLE

IN VITRO CYTOTOXICITY POTENTIAL OF ETHANOL EXTRACT OF *SYZYGIUM SAMARANGENSE* (Wt.)

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ABSTRACT

The present investigation is carried out to study the *invitro* cytotoxicity of ethanol extract of *Syzygium samarangense* leaves on HeLa cell line by using MTT assay. Ethanol extract of *S. samarangense* showed concentration dependent activity on HeLa cell line with IC50 value of 40.5 µg/ml which shows that ethanol extract of *S. samarangense* posses significant cytoxicity.Moreover the preliminary phytochemical screening showed the presence of fatty acids, alkaloids, flavonoids, terphenoids, saponins, tannins and steroids which are responsible for its cytotoxicity. There are only a few reports are available for cytotoxicity of ethanol extract of *S. samarangense*.

Keywords: *S. samarangense*, *In vitro* cytotoxicity, HeLa cell and MTT assay.

1. INTRODUCTION

Plant based products are widely used for the various treatments from the early stages of human civilization (1). The medicinal plants are herbs that contain phyto active components which are used for disease remedies could be in any usual forms such as infusions, decoctions, tinctures, syrups, infused oils, essential oils, ointments and creams (2-4). Now a days due to various forms of pollutions and life styles of human much number of diseases are out breaking, especially cancer, is widely identified and cured by many new drugs, but these are having enormous side effects and high cost. So our aim of this present investigation is to evaluate *in vitro* cytotoxic effect of ethanol extract of *Syzygium samarangense* (Wt.) Walp. that belongs to the family *Myrtaceae.* Its native is Fiji, India, Indonesia, and Malaysia. The common names of *S. samarangense* are wax apple, love apple, Java apple. The genus comprises about 1200 to 1800 species (5) and has a native range that extends from Africa and Madagascar through Southern Asia east through the Pacific. Its highest level of diversity occurs from Malaysia to North Eastern Australia. The *Syzygium* species were reported to possess various pharmacological properties viz. anti diabetic, antifungal, anti-inflammatory, antibacterial, antioxidant, anti hyperlipidemic and anti proleferative activities. (6-10). From the literature survey, there are few reports are available for the *in vitro* cytotoxicity of the ethanol extract of *S. samarangenese* leaves. Hence the present study is designed to evaluate the cytotoxicity study and preliminary phytochemical screening of *S. samarangense* leaves.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

Syzygium samarangense of plant family *Myrtaceae* was collected from area near Palani, Tamil Nadu, South India (Geographic coordinates of Palani, India Latitude: 10°27′01″N, Longitude: 77°31′15″ E Elevation above sea level: 328 m =1076ft). The plant sample was authenticated from Dr.Logamadevi Assitant Professor, Department of Botany and the voucher specimens have been kept for further reference.

2.2. Extraction Process

The leaves of *S. samarangense* were dried in shade for 10 days and tightly packed for further process. Air dried leaves of *S. samarangense* was chopped into small pieces. The coarse material was subjected to maceration and Soxhlet extraction by using different solvents. Solvents are used based on their increasing order of polarity i.e. Petroleum ether, Acetone and Ethanol. Solvent are used based on their increasing of polarity. The extract was subjected to vacuum distillation and was concentrated to yield brownish residues of 80g.

2.3. Extraction process

The 300 g of shade dried leaves of *S.samarangense* was extracted with Petroleum ether using Soxhlet apparatus. The extract was subjected to vacuum distillation and was concentrated to yield a green residue of (1.3) g. The defatted plant leaves of *S. samarangense* was again extracted with acetone and ethanol.

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2.4. Preliminary phytochemical screening

Petroleum ether, ethanol and acetone extracts of *S. samarangense* was subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present in it. The following tests were evaluated to identify the phytochemicals present.

2.4.1. Test for steroids and terpenoids

Libermann – Burchard's regent test

The extract (50 mg) was dissolved in 2 ml acetic anhydride. To this one or two drops of concentrated H_2SO_4 were added slowly along the sides of the test tube. An array of colour change showed the presence of steroids and triterpenoids.

2.4.2. Test for flavonoids

Shinoda Test

The extract mixed with few ml of alcohol was heated with magnesium and then con. HCl was added under cooling. Appearance of pink colour indicates the presence offlavonoids.

2.4.3. Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

2.4.4. Test for phenols and tannins

Small quantity of 50% alcoholic extract was dissolved in water and 5% ferric chloride solution. Appearance of blue colour with ferric chloride indicates the presence of tannins and phenols.

2.4.5. Detection of Alkaloids

The extracts were dissolved in dil. H2SO4 and filtered. The filtrate was treated with Dragendroff' reagent, the appearance of orange brown precipitate indicated the presence of alkaloids.

2.5. In vitro cytotoxicity by MTT Assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinatedehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hour of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37˚C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula. % cell Inhibition = 100- Abs (sample)/Abs (control) x100. Nonlinear regression graph was plotted between % Cell inhibition and Log10 concentration and IC50 was determined using Graph Pad Prism software.

3. RESULT AND DISCUSSION

3.1. Preliminary phytochemical screening

Petroleum ether, ethanol & acetone extracts of *S. samarangense* were subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present in it. Which are shown in table.1

Table 1. Phytochemical constituents of *S. samarangense.*

S. No	COMPOUNDS	PET ETHER	ACETONE	ETHANOL
1	Fatty acids	+ + +	_	_
2	Alkaloids	+	+ + +	+ +
3	Terpenpoids	+ + +	+	+ +
4	Flavonoids	_	+ + +	+ +
5	Steroids	+ + +	_	_
6	Saponins	+ +	+ + +	+ +
7	Carbohydrates	+ +	+ +	_
8	Phenolics	_	+ + +	_

-Indicates absence of compounds, + indicates smaller amount of compounds

+ + indicates high amount of compounds, + + +indicates very high amount of compounds

3.2. In vitro cytotoxicity study

MTT assay is widely used assay for plants and plant based products which measures cell viability of cancer cell lines.

Table 2. IC_{50} values for Ethanol extract of *S. samarangense* leaves.

Name of the extract	IC_{50} µg/ml	Name of the cell line
Ethanol	40.5 µg/ml	HeLa

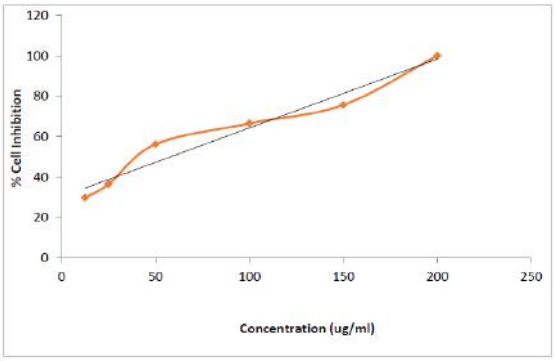

Fig. 1. % of cell inhibition at various concentration of Ethanol extract of *S. samarangense* leaves.

In this investigation ethanol extract of *S. samarangense* leaves was evaluated for *in-vitro* cytotoxicity studies using human breast cancer (HeLa). The viability of cancer cells after incubation with different concentrations of *S. samarangense* leaves (12.5, 25, 50, 100, 150 and 200 µg/ml) affected the viability of human breast cancer cell line HeLa in a dose dependent pattern and the IC 50 values was determined as 40.5 µg/ml. The results are shown in Table 2.

4. CONCLUSION

In the present study, the ethanol extract of *S. samarangense* leaves was evaluated for its phytochemical screening and *in vitro* cytotoxicity effects. It was found that the cell viability of cancer cell line was dose dependent pattern with IC50 µg/ml. This may be due to the presence of flavonoids, steroids and alkaloids. Further studies are in progress.

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RESEARCH ARTICLE

PROXIMATE COMPOSITION, NUTRITIVE SUBSTANCE AND PHYTOCHEMICAL EVALUATION OF WILD EDIBLE FRUITS OF VELLIANGIRI HILLS OF COIMBATORE DISTRICT

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ABSTRACT

Fruits contribute significantly to the food security of the society especially in terms of vitamins and micronutrients. Numerous wild edible fruits from various families distributed in Poondi, Velliangiri hills, Coimbatore were assessed the Malasar tribal people consume these fruits as a natural source of food supplement. The five wild edible fruits *viz*., *Zizipus jujuba* Mill. *Z.oenoplia* Mill. (Rhamnaceae), *Limonia acidissimia* L. (Rutaceae), *Phyllanthus emblica* L. (Euphorbiaceae) and *Ficus racemosa* L. (Moraceae) were assessed for their mineral and phytochemical contents. In which the underutilized fruits possesses high nutritional and medicinal properties for the ethanolic extracts. The macro and micro elements and their constituents explored that Fe was abundant in all the edible fruit species. while *Z.jujuba* was observed to contain high Fe content than those of others. Qualitative phytochemical screening also revealed the presence of phenolics, alkaloids, flavanoids and terpenoids. In quantitative phytochemical estimations also phenolic and tannin concentration was found to be high in *F. racemosa* while *L.acidissimia* fruit depicted maximum total flavonoid content. The nutritional and phytochemical composition of fruits indicates that, these neglected wild edible fruits can be a valuable source of nutrients under famine conditions and high levels of some vitamins can be used to prevent diseases.

Keywords: Edible fruits, minerals, nutrients, phytochemical, food.

1. INTRODUCTION

Many wild plants serve as alternatives to staple foods during period of food deficit and are valuable supplements for a nutritionally balanced diet. Wild edible fruits are one of the primary alternative source of income for tribal communities and fundamentally used for domestication (1). Wild fruits are generally used as raw or processed, which help to compensate the day-to-day requirement of calories. Wild fruits play a significant role in human nutrition, especially as sources of carbohydrates, proteins, vitamins, minerals, dietary fiber and enormous medicinal potential (2-5). Natural products have high fiber content which serves as a source of defensive properties, because of their cell reinforcement action. In the most recent decades, unique consideration has been paid towards palatable plants, particularly those that are wealthy in optional metabolites. There has been an expanding enthusiasm for cancer preventinh action of such phytochemicals (6). Therefore investigation of wild palatable organic products is essential to recognize the potential sources which could be used as elective sustenance.

In present examination investigation on the dietary status of five wild organic products viz. *Zizipus jujuba* Mill. *Z.oenoplia* Mill. (Rhamnaceae), *Limonia acidissimia* L. (Rutaceae), *Phyllanthus emblica* L. (Euphorbiaceae) and *Ficus racemosa* L. (Moraceae) evidently by the Malasar tribes individuals of Velliangiri hills, Western Ghats, Coimbatore. According to *Z. jujuba* is utilized generally as a tonic and now and again as sleep inducing narcotic. Moreover, there are ponders that had been done to test its anxiolytic, anticancer, hostile to hypersensitivity, subjective and wound mending properties (7,8). Likewise, *Z.oenoplia* is one of the society home grown plants accepted to have some pharmacological properties as blood purifier, febrifuge, stomach torment executioner, and so forth.(9,10). While, *Limonia* fruits are refrigerant, stomachic, stimulant, tonic to liver and lungs, fixes hack, hiccup and useful for asthma and leucorrhoea (11). The customary utilization of *P. emblica* is viewed as incredibly helpful in improving assimilation, decreasing clogging, diminishing fever, postponing maturing and expanding healthspan (12,13) just as *F. racemosa* utilized as a poultice in provocative bubbles and is respected to be powerful in the treatment of heaps, dysentry, asthma and urinary maladies (14). The principle focus of this exploration was to discover the healthful potential and phytochemical of these wild edible fruits.

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2. MATERIALS AND METHODS

2.1. The Malasar in Velliangiri hills

Previously described ecosystems and aboriginal communities for area of study. The Malasars (etymology in tamil – mala = hill; saras = people who live in and depend on the hills) are an aboriginal community who reside in the forest of the Velliangiri holy hills (15,16). They are traditionally hunter gathers. In the Velliangiri hills their settlements were situated near Poondi. The Malasars are considered the 'lords of the hills' (17, 18) stated that there is no information regarding the origin and early history of the Malasars.

2.2. Study area

The Velliangiri hills forms a major range in the Western Ghats that is rich in biodiversity and largely untouched by development because of its cultural and religious importance (19). In a floristic investigation that revealed considerable diversity 1715 species of angiosperms including 439 endemics within the Velliangiri hills (15, 16). The study site (longitude 6° 40' to 7° 10' E and latitude 10° 55' to 11° 10' N) is located within the Velliangiri holy hills, which forms a major range in the Western Ghats in the Nilgiri Biosphere Reserve. The research was conducted among seven hills with altitudes ranging from 520 m – 1840 m, which is bordered by the Palghat district of Kerala on the western boundary, the plains of Coimbatore district to the east, the Nilgiri Mountains to the north, and the Siruvani hills on the southern boundary. The annual rainfall is quite variable in the hills (500 mm– 7000 mm) with temperatures ranging from 0°C during winter to 41°C in the summer (20).

2.3. Plant materials

Five medicinal wild edible fruits namely viz. *Zizipus jujuba* Mill. *Z.oenoplia* Mill. (Rhamnaceae), *Limonia acidissimia* L. (Rutaceae), *Phyllanthus emblica* L. (Euphorbiaceae) and *Ficus racemosa* L. (Moraceae) were collected from Velliangiri slopes, Western Ghats, Coimbatore. Botanical identification and authentication were performed at Department of Botany, Kongunadu Arts and Science College. The identification and the medicinal uses of the fruits were shown in Figure 1.

2.4. Powder preparation

The different edible fruits were collected, washed thoroughly with fresh running water, dried under shade with room temperature (25±1) °C for a few weeks and coarsely powdered in a blender. The powdered fruit samples were separately kept in an airtight container until use (21).

2.5. Estimation of minerals in fruit material

For mineral content estimation 100 g of fine powdered sample of each fruit was digested using concentrated HNO3 and HClO4 . The digested samples were used for elemental analysis. Iron (Fe), phosphorus (P), Magnesium (Mg) and Zinc (Zn) were determined using Atomic Absorption Spectrophotometer and powdered form of fruit sample was used for estimation of Potassium (K) and Calcium (Ca) using Flame photometer (22).

3. IN VITRO STUDIES

3.1. Preliminary qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in various solvent extracts of leaf, stem, flower and fruit parts of *E.munronii* and *E.tuberculatus* (23,24).

3.2. Quantitative phytochemical analysis Total phenolics and tannins

The total phenolic content of plant extracts was determined using Folin-ciocalteu reagent according to the procedure described by (25). In this method, 20 µg of the extract (dissolved in the respective solvent) was taken in a test tube and made up to the volume of 1.0 mL with distilled water. Then 0.5 mL of freshly prepared Folin-ciocalteu phenol reagent (1:1 with water) and 2.5 mL of 20% sodium carbonate solution were added sequentially in each tube. The mixtures were agitated and left in the dark at laboratory temperature for 40 min for the development of colour. The absorbance was recorded at 725 nm against the reagent blank using a Shimadzu – UV-160 spectrophotometer (Japan). A calibration curve of gallic acid was constructed, and linearity was obtained in the range of 10-50 µg/ mL. Using the standard curve, the total phenol content of the extract was calculated and expressed as gallic acid equivalent (GAE) mg/ g extract. Using the same extract, tannin content was estimated after treatment with polyvinyl polypyrrolidone (PVPP) as described by (26). One hundred milligrams of PVPP was weighed in a 100 ×12 mm test tube and to this, 1.0 mL distilled water and 1.0 mL of tannin containing phenolic extract was added. The contents were vortexed and kept at 4ºC for 15 min. Then the sample was centrifuged (5000 rpm for 10 min at laboratory temperature) and the supernatant was collected. This supernatant has

only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured, as monitored above and expressed as the content of free phenolics on a dry matter basis. From the above results, the tannin content of the extract was calculated as follows:

Tannin (mg GAE/ g extract) = Total phenolics (mg GAE/ g extract) – Free phenolics (mg GAE/ g extract)

3.3. Total flavonoid content

The total flavonoid content was determined spectrophotometrically using the method adopted by (27). 0.5 mL of appropriately diluted extract solution was mixed with 2.0 mL of distilled water and subsequently with 0.15 mL of 5% sodium nitrite solution and maintained for 6 min. Then, 0.15 mL of 10% aluminium chloride solution was added and allowed to stand for 6 min, and finally 2.0 mL of 4% sodium hydroxide solution was added. Final volume of the contents was made up to 5.0 mL with distilled water and were mixed thoroughly. After 15 min of incubation at laboratory temperature, the absorbance was determined against blank at 510 nm. The total flavonoid content was determined using a standard curve with rutin. The mean of the three values were expressed as milligrams of rutin equivalents (mg RE)/ g extract on a dry weight basis.

4. STATISTICAL ANALYSIS

The results were expressed as the averages of three replications. The data was subjected to ANOVA. The graphs were drown by Microsoft excel 2010.

A). *Zizipus jujuba* Mill.(Rhamnaceae) B). *Zizipus oenoplia* Mill. (Rhamnaceae)

C). *Limonia acidissimia* L. (Rutaceae) D). *Phyllanthus emblica* L. (Euphorbiaceae)

E). *Ficus racemosa* L. (Moraceae)

5. Results and Discussion

5.1. Minerals quantification

The mineral creations of condiments are appeared Table 1. The consequences of the investigations were built up to give supplement esteems per 100 grams of utilized bit of dried weight. Mineral components were found to fluctuate broadly relying upon the diverse flavors. As indicated by results, Ca, K, Mg and Fe substance were high in all the wild consumable edible fruits. In addition of P and Zn components were found in a comparable range for all organic products. As per (28) assurance of overwhelming metals in ecological, natural and nourishment tests has drawn a noteworthy consideration due to the lethal and wholesome impacts of these components or their compounds.

In this work, Fe was plenteous in all edible fruit species. Then again the dimension of Fe in *Z.jujuba* was observed to be higher than those of others (7.11 mg/100 g). The largest amounts of Fe were additionally found in *P. emblica, F. racemosa, Z.oenoplia* and *L.acidissima* to be 4.26 mg/100 g, 2.68 mg/100 g, 0.823 mg/100 g and 0.32 mg/100 g separately. P substance of *P. emblica* (0.019 mg/100 g) was observed to be fundamentally the same as those of different fruit species. These distinctions may be because of development conditions, hereditary elements, land varieties and logical strategies (29,28).

5.2. Qualitative phytochemical analysis

Phytochemicals are the bioactive principles produced by fruits in its various species. These phytochemicals have great potentialities in drug discovery for various diseases (30). The phytochemicals like alkaloid, phenols, flavonoids, steroids and tannins compounds are remedy to cure diseases and fight against different kinds of pathogens, as medicine (31). In the current investigation, the qualitative phytochemical screening was conducted for the different fruits and

ethanolic extracts of viz. *Zizipus jujuba* Mill. *Z.oenoplia* Mill. (Rhamnaceae), *Limonia acidissimia* L. (Rutaceae), *Phyllanthus emblica* L. (Euphorbiaceae) and *Ficus racemosa* L. (Moraceae) and it revealed the presence of a diverse class of phytochemical constituents, including alkaloids, flavonoids, phenols, tannins, triterpinoids and steroids (Table 2). However, alkaloids and phenols were found to be present in trace amount while phenols, tannins, triterpinoids, steroids and flavanoids were completely low in all the studied fruit species.

5.3. Quantitative phytochemical analysis

Diets rich in fruits have been considered as excellent sources of antioxidants (32). The TPC concentration and the antioxidant capacity of the edible fruits depicted (Figure.2). The TPC was evaluated using the Folin-Ciocalteu assay, which is considered a fast and reliable way to quantify phenolics in foods (33). The highest TPC concentration was found in *F. racemosa* (85 mg GAE/g), followed by *P. emblica* (72 mg GAE/g) *Z.oenoplia* (65 mg GAE/g) *L.acidissima* (52 mg GAE/g) and *Z.jujuba* (47 mg GAE/g) which presented the lowest TPC. The phenolic content generally correlates with antioxidant capacity for various types of fruits (34, 35). In this study, the amount of tannin content was determined and it was found that the wild edible fruit species manifested significant content (Fig.2). In the quantification of tannin content of *F. racemosa* fruit provided highest tannin content of 32 mg GAE/g whereas lowest content was determined by of *Z.jujuba* (15 mg GAE/g). However, (36) the recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances. Apart from this, *L.acidissima* fruit depicted maximum total flavonoid content (61 mg RE/g) while, the edible fruit of *F. racemosa* registered very low content (12 mg RE/g). While, (37) flavonoids are also abundantly found in foods and beverages of plant origin, such fruits hence they are termed as dietary flavonoids.

Table 1. Proximate composition of various wild edible fruits.

Samples	K	Ca	Mg	Fe	Zn	P
Z.jujuba	0.012	0.151	0.039	7.11	0.105	0.049
Z.oenoplia	0.023	0.103	0.192	0.823	0.067	0.025
L.acidissima	0.185	0.096	0.147	0.32	0.072	0.054
P. emblica	0.104	0.176	0.154	4.26	0.056	0.019
F. racemosa	0.278	0.119	0.09	2.68	0.126	0.095

*Values were expressed as mg/g dried samples.

Table 2. Qualitative phytochemical analysis of ethanolic extracts of different wild edible fruits.

Tests	*Z .jujuba*	*Z. oenoplia*	*L. acidissima*	*P. emblica*	*F. racemosa*
Alkaloids	+++	+++	+++	+++	+++
Flavonoids	+++	+++	+	+++	+
Phenols	+++	+++	+++	+++	+++
Terpinoids	++	++	++	+++	+
Tannins	++	++	+	++	+
Steroids	++	+	+++	++	++

*Legend: +++ (Much abundant), ++ (less abundant), + (minute)

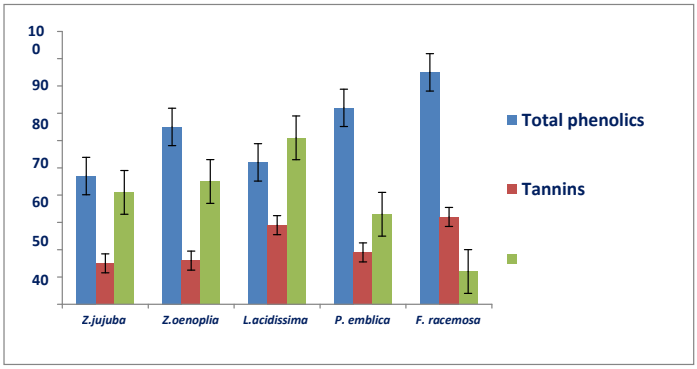

Fig 2. Total phenolics, tannins and flavonoid content in ethanolic extracts of wild edible fruits.

6. CONCLUSION

The results of this study demonstrated significant differences found in total phenolic, tannins and flavonoid content of different wild edible fruit species, and also in terms of mineral compounds. This study is meant to be a contribution to the characterization of chemical extracts of wild flora fruits that are traditionally used for medicinal applications. Fruits that were studied may have great potential for food production as sources of bioactive compounds such as phenolic compounds and minerals, and also for food supplements or functional foods.

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RESEARCH ARTICLE

OBSERVATIONS ON THE ENDEMIC TAXA OF VELLIANGIRI HILLS, A PART OF SOUTHERN WESTERN GHATS, INDIA

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ABSTRACT

The present exploration based on the floristic studies carried out shows that there are about 121 endemic taxa of flowering plants in Velliangiri hills belonging to 34 families have been recorded and are listed. Out of the plants listed, the dominant families are Asteraceae, Lamiaceae, Fabaceae and Orchidaceae. From the list of the endemic plants in the study, it is indicated that the phylo-genetically advanced families such as Orchidaceae, Asteraceae, and Lamiaceae are the most diversified families on the basis of their number of genera and species. The herbs and shrubs are better represented than the trees in the list. A large number of endemic taxa (about 20 species) are known only by their type collection, which could not be recollected or relocated even after repeated field explorations. It seems that either these taxa are vanished from their localities or alternatively, that species misidentification has occurred.

Keywords: Endemic taxa, Velliangiri hills, exploration, assessment of status.

1. INTRODUCTION

The Velliangiri hills, the horse-shoe-shaped range of hills which are situated exactly to the west of Coimbatore town and comes under Boluvampatti reserve forests (1). At the base of this hill and facing east is Siruvani foot. Here there are settling tanks and these collect water from a narrow channel drawn from Muthukolam, which is the main source of water supply for Coimbatore town and is situated in a ravine at the top of a group of hills. The study site Velliangiri hills which forms a major hill range in the Western Ghats that is rich in biodiversity and largely untouched by development because of its cultural and religious importance (2). It is situated to the western boundary of Palghat district of Kerala, the plains of Coimbatore district of Tamil Nadu to the east, the Nilgiri Mountains to the north. The area extended approximately 48 sq. km. and consists of seven hillocks with different altitudes and micro-topography. It lies between 76° 40' and 77° 10' E longitude and 10° 55' and 11° 10' N latitude. The altitude ranges from 520 to 1840 m above MSL (Fig. 1)

Velliangiri hills is popularly known as "Thenkailaya malai" (in Tamil) which means the holy hills of Southern India. The Velliangiri Andavar temple and the cave of "Shivalinga" is situated at the peak of this hill. The season of pilgrims visiting to this temple is the month of March to May of each year and on moon days of each month. The pilgrims visit the hill peak temple bare foot as they believe that the holiness will lose if they wear shoes and chapels. The devotees visit this temple by walk through the thick jungles, wet evergreen forests, shola forests and grassland vegetation. The pilgrims start walking up the hill early in the morning and climb down before dark (night).

2. GEOLOGY, ROCK AND SOIL

The hills have come in the existence due to the upright by block faulting in the cretaceous and Miocene periods and consist of Precambrian Archean crystalline hard rocks of charmokits belonging to the Nilgiri gneiss. The gneiss is finely foliated and is composed of quartz, felspar, and biotite (black mica) with an occasional admixture of garnet (3). Though laterite in its pure form is not met on the hills, the rocks do undergo a change akin to laterite metamorphosis resulting in the formation of soil varying from pale yellow to red colour in the form of a sandy loam. The soil type of this hill is red, loamy, acidic and ferruginous. Along the foot hills, the soil is reddish with irregular galleries filled with yellow clay running through its mass and it has the property of hardening on exposure to the air. The scrub jungle region possess dry rocky soil whereas in the evergreen as well as the grassland regions the soil becomes dark humus and fertile (4). In the plains where the crops are cultivated, the black cotton soil and a sandy soil along the Noyil River.

3. CLIMATE AND RAIN FALL

The difference in elevation between the plains and the ghats makes appreciable variations in their climatic conditions. As, the area lying on the

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eastern side, it receives more wind during the South-west monsoon from June to September and during this period climate is cool and pleasant. The North-east monsoon brings most of the annual rainfall and the climate regions cool from November to February. The climate is cool and pleasant for the major part of the year except during the month of March to May when it is hot and dry. The temperature of the hills ranging from 2°C during winter and upto 41°C during summer. Temperature at the foot hills ranges from 24°C to 38°C and night temperatures 18°C to 29°C and the mean annual humidity is 51% (5). The prevailing winds are from the west and south-west during April to September and from the east and north-east from October to March. The western sea-breeze blows slightly in the evenings from the month of March onwards developing into the South-west monsoon about the beginning of June.

The high mountains in the Western Ghats keep study site away from South-west monsoon and the small showers received during June and July are only the portion of the South-west monsoon escaping through the narrow gaps of the Western Ghats. As the hill ranges open eastwards the North-east monsoon is received properly. Venkateswara Ayyar (1939) has given the average rainfall of this area based on the observations made at the Iruttupallam Office of the Forest Department. The average annual rainfall of this hills is 3500 mm at the foot hills and 4500 mm at the peak and the amount of rainfall increases with increase of altitude. The area is subjected to both South-West and North-East monsoon and the rainfall during South-West monsoon is heavy and usually starts by the middle of May and lasts up to August. The configuration of mountain ranges, the topographical variations, angles of slopes and the altitudinal levels at different hills generally favour the precipitation during south-west (June-August) and north-east (October-December) monsoon periods.

Fig. 1. Study area.

Fig. 2. An overview the species in Velliangiri Hills.

Table 1. List of species in Velliangiri Hills.

Sl. No.	Name of the family with species	Habit	Phenology	Region	Reference	Specimen No.
	Ranunculaceae					
1	*Clematis wightiana*		February – March			8211
2	*Ranunculus wallichianus*	Climber	June – October	WG	Matthew, 1999 (6)	8212
	Berberidaceae					
3	*Mahonia leschenaultia*	Tree	May – December	WG	Nayar, 1996 (7)	8213
	Polygalaceae					
4	*Polygala jacobii*	Herb	May – December	TN	Ahmedullah & Nayar, 1986 (8)	8163
	Clusiaceae					
5	*Mesua ferrea*	Tree	March – October	SI	Nair & Henry, 1983 (9)	8216
	Bombacaceae					
6	*Bombax insigne*	Tree	November – March	TN	Jain & Sastry, 1984 (10)	8217
	Elaeocarpaceae					
7	*Elaeocarpus recurvatus*	Tree	February – May	SWG	Nayar & Sastry, 2000 (11)	8191
	Oxalidaceae					
8	*Biophytum longipedunculatum*	Herb	December – April	SI	Nair & Henry, 1983 (9)	8222
	Balsaminaceae					
9	*Impatiens clavicornu*		June – October		Nair & Henry, 1983	8168

		Herb		WG	(9)	
10	*I. inconspicua*		September – December			8154
11	*I. leschenaultii*	Shrub	April – December		Matthew, 1999 (6)	8148
12	*I. phoenicea*	Herb	September – October	SWG	Ahmedullah & Nayar, 1999 (8)	8144
13	*I. viscida*	Herb	April – December		Nair & Henry, 1983 (9)	8141
	Rutaceae					
14	*Atalantia wightii*	Shrub	March – June	PI	Matthew, 1999 (6)	8222
15	*Melicope indica*	Tree	September – December	SWG	Nayar, 1996 (7)	8223
	Meliaceae					
16	*Aglaia indica*	Tree	May – June	SWG	Nayar, 1996 (7)	8224
	Vitaceae					
17	*Ampelocissus araneosa*	Climber	July – December	WG	Nayar, 1996 (7)	8227
18	*Cayratia pedata*	Climber	March – August	TN	Nayar &Sastry, 1987 (11)	8228
	Sapindaceae					
19	*Allophylus serrulatus*	Shrub	July – October			8229
20	*Buchanania lanceolata*	Tree	November – March	WG	Ahmedullah & Nayar, 1987 (8)	8230
	Fabaceae					
21	*Crotalaria fysonii*		September – December	WG		8232
22	*C. globosa*		November – March	SWG		8193
23	*C. hirsuta*		March - April	WG		8172
24	*C. longipes*		November – January			8157
25	*C. obtecta*		December – March	SWG		8145
26	*C. scabrella*	Herb	November – March	WG	Nayar, 1996 (7)	8140
27	*Indigofera uniflora*		September – December	SWG	Ahmedullah & Nayar, 1987 (8)	8233
28	*Rhynchosia filipes*	Climber	November – March	SI	Ahmedullah & Nayar, 1987 (8)	8235
29	*Tephrosia roxburghiana*	Herb	July – September	SWG	Nayar, 1996 (7)	8236
	Rosaceae					
30	*Rubus racemosus*	Climber	December – May	SWG	Ahmedullah & Nayar, 1987 (8)	8237
	Crassulaceae					
31	*Kalanchoe olivacea*	Shrub	December – March	TN	Nayar & Sastry, 1987 (11)	8238
	Myrtaceae					
32	*Eugenia indica*		March – May		Nayar, 1996 (7)	8239
33	*Syzygium benthamianum*		December – February	SWG		8240
34	*S. densiflorum*	Tree	April – June		Ahmedullah & Nayar, 1987 (8)	8175
35	*S. travancoricum*			WG	Nayar & Sastry, 1987 (11)	8151
	Melastomataceae					
36	*Medinilla malabarica*		September – December		Ahmedullah & Nayar, 1987 (8)	8241
37	*Memecylon lawsonii*	Shrub	September – March	WG		8242
38	*Osbeckia gracilis*				Nayar, 1996 (7)	8243
39	*O. leschnaultiana*		January – April	SWG	Ahmedullah & Nayar, 1986 (8)	8183
40	*Sonerila rotundifolia*		July – October		Nayar, 1987 (11)	8179
41	*S. versicolor*	Herb	June – December	SWG	Ahmedullah & Nayar, 1987 (8)	8162
	Apiaceae					
42	*Bupleurum distichophyllum*	Herb	June – October	SWG		8245
43	*B. plantaginifolium*		August – October		Nayar, 1996 (7)	8195
44	*Heracleum rigens*		August – September			8246
45	*H. sprengelianum*		July – November	WG		8187

46	*Pimpinella candolleana*		Aug. – October	SWG		8248
	Rubiaceae					
47	*Hedyotis hirsutissima*	Shrub	November – March	WG	Nayar & Sastry, 1987 (11)	8250
48	*H. leschenaultiana*			SWG	Nayar, 1996 (7)	8188
49	*Knoxia wightiana*			WG		8251
50	*Lasianthus parvifolius*	Shrub	December – May	WG	Ahmedullah & Nayar, 1987 (8)	8252
51	*Psychotria bisulcata*		March – July	SWG		8254
52	*P. nilgiriensis*		September – December	WG	Nayar, 1996 (7)	8180
	Asteraceae					
53	*Anaphalis aristata*		July – April	WG		8256
54	*A. beddomei*		June – October	TN	Henry et al., 1987 (12)	8198
55	*A. elliptica*		April – November	WG	Henry et al., 1987 (12)	8143
56	*A. lawii*		September – March	SI	Ahmedullah &	8173
57	*A. leptophylla*		June – October	SWG	Nayar, 1987(8)	8158
58	*A. neelgerryana*	Herb	July – October	WG	Henry et al., 1987(12)	8150
59	*A. wightiana*		September – June	SWG	Ahmedullah & Nayar, 1987(8)	8146
60	*Blumea wightiana*		January – November	SWG	Nayar, 1996 (7)	8257
61	*Gynura nitida*		January – December	WG	Ahmedullah & Nayar, 1987 (8)	8258
62	*Helichrysum wightii*		November – April		Henry et al., 1987 (12)	8259
63	*Sonecio lavandulaefolius*		October – December	SWG	Ahmedullah &	8260
64	*Vernonia conyzoides*		September – March	SWG	Nayar, 1987(8)	8261
65	*V. travancorica*	Tree	February – July	WG	Henry et al., 1987(12)	8174
	Ebenaceae					
66	*Diospyros bourdillonii*	Tree	March – December	SWG	Nayar, 1996(7)	8262
	Symplocaceae					
67	*Symplocos macrophylla*	Tree	February – May	SWG	Ahmedullah & Nayar, 1987(8)	8263
68	*S. racemosa*		December – February	WG	Henry et al., 1987 (12)	8176
	Oleaceae					
69	*Jasminum rottlerianum*	Climber	January – June	PI	Henry et al., 1987 (12)	8264
70	*Ligustrum perrottetii*	Tree	February – June	WG	Nayar, 1996(7)	8265
	Asclepiadaceae					
71	*Ceropegia intermedia*		June – December	SWG	Ahmedullah &	8266
72	*Sarcostemma intermedium*	Climber	Aug. – December	PI	Nayar, 1987 (8)	8267
	Gentianaceae					
73	*Exacum wightianum*		April – July			8268
74	*Swertia beddomei*		November – April	SWG	Ahmedullah &	8269
75	*S. corymbosa*		October – December	WG	Nayar, 1987 (8)	8177
76	*S. densifolia*		September – January	SI	Henry et al., 1989(12)	8160
77	*S. lawii*	Herb	November – January	WG	Ahmedullah &	8152
78	*S. minor*		August – September	WG	Nayar, 1987(8)	8147
	Gesneriaceae					
79	*Didymocarpus gambleanus*	Herb	April – August	SWG	Ahmedullah & Nayar, 1987 (8)	8273
	Acanthaceae					
80	*Andrographis lobelioides*		September – December			8274
81	*Barleria acuminata*	Herb	December – March		Ahmedullah &	8275

82	*Justicia wynaadensis*		November – March		Nayar, 1987 (8)	8276
83	*Strobilanthes foliosus*	Shrub	October – December	WG		8277
84	*S. kunthianus*	Shrub	September – January			8178
85	*S. lawsonii*	Shrub	July – September		Nayar, 1996 (7)	8161
	Lamiaceae					
86	*Anisochilus dysophylloides*	Herb	December – March		Nayar, 1996 (7)	8278
87	*Isodon nilgherricus*		October – February			8279
88	*Leucas lancifolia*	Shrub		SWG		8280
89	*L. pubescens*		June – August		Ahmedullah &	8185
90	*L. ternifolia*	Herb	July – February		Nayar, 1987 (8)	8167
91	*Plectranthus bishopianus*	Shrub	September – December	WG	Nayar & Sastry, 1990 (11)	8281
92	*P. subincisus*	Shrub	September – November	TN	Henry et al., 1987 (12)	8165
93	*P. urticifolius*	Herb	October – January			8153
94	*Pogostemon atropurpureus*	Shrub	February – May		Nayar, 1996(7)	8282
95	*P. mollis*	Herb	October – February			8181
96	*P. nilagiricus*	Shrub	January – April	WG	Nayar & Sastry, 1990 (11)	8164
97	*P.vestitus*		November – January		Nayar, 1996 (7)	8215
98	*Scutellaria colebrookiana*	Herb	October – December		Ahmedullah & Nayar, 1987 (8)	8283
	Lauraceae					
99	*Actinodaphne bourdillonii*		April – August		Ahmedullah &	8284
100	*Cinnamomum perrottetii*		February – May		Nayar, 1987 (8)	8285
101	*C. sulphuratum*		March – August	WG		8194
102	*Cryptocarya bourdillonii*	Tree	April – December		Henry et al., 1987 (12)	8286
103	*Litsea floribunda*		December – April	SWG	Nayar, 1996(7)	8287
104	*L. insignis*		March – July	WG	Henry et al., 1987 (12)	8184
	Euphorbiaceae					
105	*Bridelia crenulata*		May – September		Nayar, 1996 (7)	8289
106	*Glochidion bourdillonii*	Tree	February – July	WG	Ahmedullah & Nayar, 1987 (8)	8290
	Orchidaceae					
107	*Calanthe triplicata*		October – December	WG	Jain & Rao, 1983 (13)	8294
108	*Disperis neilgherrensis*		May – June	SI	Sarkar, 1995 (14)	8295
109	*Habenaria elliptica*		August – November	WG	Ahmedullah & Nayar, 1987 (8)	8297
110	*H. longicorniculata*	Herb		WG	Sarkar, 1995 (14)	8189
111	*H. polyodon*			SI	Ahmedullah &	8170
112	*H. rariflora*		July – September	WG	Nayar, 1987 (8)	8156
113	*Malaxis acuminata*		June – December	WG	Sarkar, 1995 (14)	8298
	Zingiberaceae					
114	*Curcuma neilgherrensis*		April – October	SI	Henry et al., 1989	8192
115	*C. pseudomontana*	Herb	July – August	PI	(12)	8171
	Liliaceae					
116	*Lilium neilgherrense*	Herb	October – December	WG	Ahmedullah & Nayar, 1987 (8)	8303
	Eriocaulaceae					
117	*Eriocaulon robustum*		June – April		Ansari & Balakrishnan (15)	8190
	Poaceae					
118	*Arundinella mesophylla*		July – December	SWG	Henry et al., 1989 (12)	8308
119	*Digitaria tomentosa*		September – December	SI	Ahmedullah &	8311

		Herb		Nayar, 1987 (8)	
120	*Eragrostiella brachyphylla*	July – December	SI	Henry et al., 1989 (12)	8312
121	*Garnotia arundinacea*	October – February	WG	Ahmedullah & Nayar, 1987 (8)	8313

(WG: Western Ghats; SWG: Southern Western Ghats; SI: Southern India; PI: Peninsular India; TN: Tamil Nadu)

4. VEGETATION

The study area composed of Bamboo forests mixed with grasslands and evergreen forests. It also forms a thick shrub jungle extending from the foot of the mountain upto a height of 700 m. At places the thorny climbers and shrubs make the scrub jungles almost impenetrable. Above this region is the beginning of the evergreen type of vegetation interspersed by grasslands. The gentle eastward slopes of the hills support shola vegetation here and there. The shola feeds a number of tributes of the Noyil River (Subramanyam, 1959). The observations made on the vegetation of the area and showed that the forest types of (i) southern tropical thorn forests (scrub jungles), (ii) tropical dry deciduous forests, (iii) tropical wet evergreen forests, (iv) temperate forests (sholas) and (v) southern montane humid grasslands as described by Champion and Seth (16). The moist deciduous forests and wet evergreen vegetation are the most dominant habitats, compared with semi-evergreen and grassland vegetation, which are restricted to a few patches at higher elevations and along streams. The forests of this area are subjected to extreme biotic influences and extensive areas are planted with Eucalyptus, Teak, Bombax, etc. The natural regeneration of trees in these forests is very poor which may be due to excessive grazing and other biotic influences. The major threat for this area is human interference during festival seasons. The devotees cut and burn many plants to keep themselves warm during the cold winter times. Hence, the indiscriminate collection of wild plants leads to extinction from the study area.

5. OBSERVATIONS

The present observations relate mainly to the endemic taxa in the Velliangiri hills and all the plants listed below have been carefully observed in the field and collected on the spot (Fig. 2). The classification of Bentham and Hooker is followed and the species under each family are arranged in an alphabetical manner and presented in the below table. For each species the following data are given: botanical name with family, habit, phenology, region of occurrence, reference for thee endemic status and finally the field/collection number. Every attempt has been made to bring the nomenclature up-to-date and the following International Plant Names Index (IPNI) websites.

Out of the plants listed, the dominant families are Asteraceae, Lamiaceae, Fabaceae and Orchidaceae. It was noticed from the list that the Asteraceae very well represented with the largest number of genera and species, the next in order was Lamiaceae and Fabaceae. Among the other families which have five and more species are Orchidaceae, Melastomataceae, Rubiaceae, Gentianaceae, Acanthaceae, Lauraceae and Apiaceae. Further it was also observed from the list that the herbs, shrubs and climbers are better represented than the trees. It is proposed to conduct more field trips to this study region to bring different seasons of the year and additional lists will be published as and when data are gathered.

6. SUMMARY

Botanical exploration as undertaken by the authors in the Velliangiri hills during 2011-2014. Several field trips were made to the various locations of these forests and valleys throughout the different season of the year (four years). Plants collected during these exploration trips were processed for the Herbarium and were identified after comparing with the authentic specimens in the Madras Herbarium. A total of 121 endemic species belonging to 34 families which have been recorded are given under observations. Out of the plants listed, the dominant families are Asteraceae, Lamiaceae, Fabaceae and Orchidaceae. The herbs, shrubs and climbers are better represented than the trees in the list.

Varying topographic and climatic conditions provided the favorable conditions for survival of the plants. The rapid global change including climate fluctuation and man-made impacts are threatening their tong-term survival. The endemic plants are threatened by rapid climate change, forest fragmentation, habitat loss, on and introduction of exotic species that bring slow death of native species. Many of the earlier described/ collected taxa needs special attention to solve the taxonomic problems and conservation measures. This can be confirmed only by collecting samples

from type localities as well as different localities. Recent studies have ascertained that many taxa are endemic to Velliangiri hills and several of them are threatened. Unless proper conservation measures are taken, many of them will become extinct. In fact there are only a few historical botanical collections from the Velliangiri hills. Hence, a thorough assessment of rare, endemic and endangered species in this area is highly essential to know the actual status and conservation of the endemic species.

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RESEARCH ARTICLE

DIRECT ORGANOGENESIS OF A CRITICALLY ENDANGERED MEDICINAL LIANA, *COSCINIUM FENESTRATUM* (GAERTN.) COLEBR. (MENISPERMACEAE)

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ABSTRACT

An efficient and reproducible regeneration protocol *via* direct organogenesis has been established using nodal explants of *Coscinium fenestratum*. Nodal explants were inoculated on MS medium supplemented with BAP and TDZ at 2.0 and 1.0mg/L respectively were produced high frequency of shoots (91.01%). The elongated shoots subcultured on half strength MS medium supplemented with IBA alone at 0.6mg/L produced high frequency of roots (97.42%). Rooted plantlets were acclimatized and established in garden soil, sand and vermicompost in the ratio 1:1:1 with good survival rate under greenhouse conditions. This protocol can be efficiently used for mass multiplication and conservation of this critically endangered medicinal liana in its native habitat.

Keywords: *Coscinium fenestratum,* MS medium, BAP, TDZ, IBA.

1. INTRODUCTION

Plants with medicinal properties have been known for thousands of years and have been used as traditional medicines by the people to treat disease. Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system of developing countries including India. Medicinal plants have a great importance due to their potential uses in pharmaceutical industries. Plant tissue culture is well known biotechnological tool for the rapid propagation of medicinal plants for the purpose of commercialization and conservation (1). The species which are failed in successful propagation through seeds or lacking vegetative reproduction can be multiplied through *in vitro* regeneration by employing this technique. Greater demand for these plants especially for the purpose of food and medicine is one of the causes of their rapid depletion from primary habitats. Micropropagation offers a great potential for large scale multiplication of such useful species and subsequent exploitation and also the application of this technology provides materials required for the isolation of drugs by the pharmaceutical industries without depleting natural plant resources.

The plant, *Coscinium fenestratum* (Gaertn.) Colebr. (Menispermaceae) is widely used as a medicinal plant in many Southeast Asian countries, mainly Oorali tribes of Idukki district and Kaadar tribes of Thrissur district for fever, muscle pain, abdominal pain, inflammation, ulcers, wounds, jaundice, burns, skin diseases, snake bite poisoning and diabetic food supplements (2). It is a main source of berberine in southern part of India (3,4). It is estimated that about 114 tons of woody stem bark of this species is extracted annually from the Western Ghats. Due to these wide medicinal importances, this species is severely exploited from its wilds in the western part of Tamil Nadu, India (5). In light of this fact, the present study was aimed to carry out the *in vitro* regeneration studies in the endangered medicinal liana, *C. fenestratum*.

Fig. 1. Habit of the study species, *Coscinium fenestratum.*

2. MATERIALS AND METHODS

2.1. Plant material and surface sterilization

Healthy and immature nodal segments of the study species, *C. fenestratum* were collected from Velliangiri hills, Coimbatore district, Tamil Nadu, India is used as explant (Fig. 1). These segments were washed under running tap water followed by treatment with a surfactant, tween 20 (5% w/v) for 5 min. To eliminate the fungal contamination, the explants were treated with carbendazim (5% w/v) fungicide also for 5 min followed by 2 or 3 rinses in sterile double distilled

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water. To eliminate bacterial contamination, explants were also treated with antibiotics (5% w/v) (Ampicillin and Rifampicin) for 5 min followed by three rinses in sterile double distilled water. Furthermore, surface sterilization was carried out by dipping the explants in 0.1% mercuric chloride ($HgCl_2$) for 3 minutes followed by 3-4 rinses in sterilized double distilled water to remove traces of $HgCl_2$.

2.2. Establishment of culture and shoot regeneration

For the shoot induction, nodal segments were inoculated vertically on MS medium (6) containing different concentrations and combinations of growth regulators such as BAP (ranging from 0.5 – 3.0 mg/L), TDZ (0.5 mg/L and 1mg/L), 2,4-D (0.5mg/L) and Kn (0.5mg/L). These cultures were incubated at 25±2°C temperature under light. The percentage of explants responding, number of shoots per explant and length of the shoots can be recorded after 40 days of culture.

2.3. Rooting of in vitro derived shoots

The proliferated shoots of 2-3cm length will be excised from the culture and transferred to half strength MS medium supplemented with various concentrations of auxin (IBA and IAA ranging from 0.2 to 1.6 mg/L and NAA ranging from 0.5 to 3.0 mg/L) for root formation. After two weeks, the rooting attributes *viz.,* the per cent shoots responding for rooting, number of roots per shoot and root length will be measured.

2.4. Percentage of response

The percentage of response of explants can be calculated as per the following formula:

$$\text{Percent response} = \frac{\text{Number of explants responded}}{\text{Number of explants cultured}} \times 100$$

2.5. Hardening of plantlets in the greenhouse

Rooted shoots will be thoroughly washed to remove the adhering gel followed by dipping in 1% (w/v) fungicide, Bavistin solution for 10-15min to remove the fungal contamination. The plantlets will be transplanted to various types of sterilized potting media as detailed below:

Hardening medium composition	**Proportion of components (v/v)**
Garden soil + sand + vermicompost	1:1:1
Red soil + sand + vermicompost	1:1:1
Vermicompost + red soil	1:1
Red soil + sand	1:1
Decomposed coir waste + perlite + vermicompost	1:1:1

Twenty five plantlets per potting mixture will be tested in the green house and growth rate can be calculated after 30 days of hardening. After acclimatization, the plantlets will be exposed to natural environmental conditions.

2.6. Observations and data analysis

The cultures were regularly subcultured on fresh medium after 4-5 week interval. The experiments were repeated thrice with twenty five replicates per treatment. All the values were expressed as mean ± standard deviation (SD) of two determinations and subjected to one-way analysis of variance (ANOVA) followed by *post hoc* Duncan's multiple range test using SPSS software (version 9, SPSS Inc., Chicago, USA). $p < 0.05$ was chosen as the criterion for statistical significance.

3. RESULTS

3.1. Shoot regeneration

The response of nodal explants for direct shooting was varied according to the combinations and concentrations of the growth regulators, BAP, TDZ, 2,4-D and Kn in the MS medium. The MS medium containing the growth regulators, BAP and TDZ at 2.0 and 1.0mg/L respectively exhibiting high degree of shooting characters like per cent explants response to shoot initiation (91.01%) and number of shoots per explant (3.87 shoots/explant) and shoot length (5.92cm) (Table 1, Fig. 2(b-g)). Due to better response of node for direct shooting, the node derived shoots were taken for further studies on rooting and hardening to derive the elite plantlets.

3.2. Rooting of in vitro derived shoots

The rooting attributes such as percentage of shoot produced roots, number of roots produced per shoot and root length have been significantly varied depending on the concentration of individual supplementation of auxins like IBA, IAA and NAA in half strength MS medium for the study species, *C. fenestratum* are exhibited in Tables 2. For the node derived shoots, the highest percentage of root formation (97.42%), greater number of roots per shoot (4.78roots/shoot) and length (4.17cm) were observed in half strength MS medium supplemented with IBA alone at 0.6mg/L (Table 2, Fig. 2h). However, the rooting percentage was significantly reduced at the lower concentrations of IAA and NAA.

Table 1. Effect of different concentrations and combinations of growth regulators in the MS medium on direct shooting (shoot initiation, shoot number and shoot length) from the nodal explant, *Coscinium fenestratum.*

Growth regulator (mg/L)				Culture response (%)	Number of shoots/explant*	Shoot length (cm)*
BAP	TDZ	2,4-D	Kn			
0.5	0.5	0.0	0.0	33.10	$0.81^{d}\pm0.09$	$2.24^{d}\pm0.31$
1.0	0.5	0.0	0.0	55.26	$1.51^{c}\pm0.26$	$3.41^{c}\pm0.41$
1.5	0.5	0.0	0.0	59.15	$1.76^{c}\pm0.20$	$4.02^{b}\pm0.62$
2.0	0.5	0.0	0.0	61.98	$1.87^{b}\pm0.28$	$4.05^{b}\pm0.17$
2.5	0.5	0.0	0.0	64.12	$1.98^{b}\pm0.32$	$4.23^{b}\pm0.62$
3.0	0.5	0.0	0.0	75.11	$2.15^{b}\pm0.47$	$5.07^{b}\pm0.49$
0.5	1.0	0.0	0.0	78.46	$2.55^{b}\pm0.41$	$5.11^{ab}\pm0.36$
1.0	1.0	0.0	0.0	80.91	$2.95^{a}\pm0.11$	$5.62^{a}\pm0.43$
1.5	1.0	0.0	0.0	83.67	$3.28^{a}\pm0.39$	$5.79^{a}\pm0.31$
2.0	1.0	0.0	0.0	91.01	$3.87^{a}\pm0.51$	$5.92^{a}\pm0.56$
2.5	1.0	0.0	0.0	85.23	$3.57^{a}\pm0.58$	$5.63^{a}\pm0.83$
3.0	1.0	0.0	0.0	82.34	$3.21^{a}\pm0.53$	$5.51^{a}\pm0.71$
0.5	0.0	0.5	0.0	30.65	$0.79^{d}\pm0.11$	$1.27^{e}\pm0.11$
1.0	0.0	0.5	0.0	42.89	$1.07^{cd}\pm0.25$	$2.35^{d}\pm0.12$
1.5	0.0	0.5	0.0	46.67	$1.23^{c}\pm0.31$	$3.56^{c}\pm0.22$
2.0	0.0	0.5	0.0	52.24	$1.35^{c}\pm0.43$	$3.97^{c}\pm0.31$
2.5	0.0	0.5	0.0	56.28	$1.62^{c}\pm0.31$	$3.82^{c}\pm0.27$
3.0	0.0	0.5	0.0	48.12	$1.26^{c}\pm0.57$	$2.14^{s}\pm0.22$
0.5	0.0	0.0	0.5	24.65	$0.72^{d}\pm0.15$	$1.78^{e}\pm0.15$
1.0	0.0	0.0	0.5	29.80	$0.77^{d}\pm0.63$	$1.82^{e}\pm0.25$
1.5	0.0	0.0	0.5	35.17	$1.00^{cd}\pm0.05$	$1.96^{de}\pm0.35$
2.0	0.0	0.0	0.5	43.04	$0.97^{cd}\pm0.61$	$2.01^{de}\pm0.26$
2.5	0.0	0.0	0.5	47.24	$1.01^{cd}\pm0.33$	$2.13^{d}\pm0.43$
3.0	0.0	0.0	0.5	31.00	$0.85^{d}\pm0.18$	$2.01^{de}\pm0.31$

*Values are presented as the mean ± standard deviation (SD) of three independent experiments.
Values not sharing a common letter in a column are significantly different ($p< 0.05$).

Table 2. Effect of different concentrations and combinations of growth regulators in the half strength MS medium on rooting percentage, root number and root length after subculturing of node derived shoots of the species, *Coscinium fenestratum.*

Growth regulator (mg/L)			Culture response (%)	Number of roots / shoot*	Root length (cm)*
IBA	IAA	NAA			
0.2	0.0	0.0	76.12	$3.42^{b}\pm0.05$	$3.44^{b}\pm0.11$
0.4	0.0	0.0	90.11	$4.45^{a}\pm0.23$	$3.95^{ab}\pm0.12$
0.6	0.0	0.0	97.42	$4.78^{a}\pm0.18$	$4.17^{a}\pm0.13$
0.8	0.0	0.0	89.14	$4.14^{ab}\pm0.14$	$4.09^{a}\pm0.12$
1.0	0.0	0.0	81.12	$3.97^{ab}\pm0.18$	$3.92^{ab}\pm0.14$
1.2	0.0	0.0	72.21	$3.56^{b}\pm0.43$	$3.89^{ab}\pm0.23$
1.4	0.0	0.0	68.47	$3.23^{bc}\pm0.26$	$3.67^{b}\pm0.38$
1.6	0.0	0.0	55.09	$2.22^{c}\pm0.78$	$3.01^{bc}\pm0.87$
0.0	0.2	0.0	25.12	$0.54^{e}\pm0.23$	$1.25^{d}\pm0.34$
0.0	0.4	0.0	39.16	$1.12^{cd}\pm0.36$	$2.05^{de}\pm0.16$
0.0	0.6	0.0	55.01	$2.21^{c}\pm0.37$	$1.82^{d}\pm0.37$
0.0	0.8	0.0	61.23	$3.58^{b}\pm0.43$	$2.59^{c}\pm0.24$
0.0	1.0	0.0	62.25	$3.71^{b}\pm0.15$	$3.06^{b}\pm0.21$
0.0	1.2	0.0	55.09	$2.38^{c}\pm0.14$	$2.75^{c}\pm0.25$
0.0	1.4	0.0	51.97	$2.21^{c}\pm0.54$	$2.53^{cd}\pm0.42$
0.0	1.6	0.0	35.11	$0.91^{d}\pm0.32$	$0.84^{e}\pm0.11$

0.0	0.0	0.5	38.85	0.76^{d}±0.41	0.65^{e}±0.22
0.0	0.0	1.0	43.31	0.72^{d}±0.32	2.21^{c}±0.18
0.0	0.0	1.5	54.16	2.43^{c}±0.41	2.62^{c}±0.13
0.0	0.0	2.0	63.42	3.51^{b}±0.27	2.91^{bc}±0.28
0.0	0.0	2.5	57.12	2.63^{c}±0.11	2.56^{c}±0.17
0.0	0.0	3.0	45.05	1.15^{cd}±0.16	1.06^{de}±0.11

*Values are presented as the mean ± standard deviation (SD) of three independent experiments.
Values not sharing a common letter in a column are significantly different (p< 0.05).

Fig. 2. Stages in *in vitro* regeneration of nodal explant of *Coscinium fenestratum* through direct organogenesis.

a) - Inoculation of nodal explant on MS medium supplemented with BAP and TDZ at 2.0mg/L and 1.0mg/L respectively.
b) & c) - Initial stage of shoot formation on MS medium supplemented with BAP and TDZ at 2.0mg/L and 1.0mg/L respectively.
d), e), f) & g) - Effective shoot formation on MS medium fortified with BAP and TDZ at 2.0mg/L and 1.0mg/L respectively.
h) - Root induction of *in vitro* derived shoots on MS medium containing IBA alone at 0.6mg/L.
i) - An acclimatized potted plantlets in the hardening medium composed by garden soil, sand and vermicompost in the ratio of 1:1:1 by volume.

3.3. Acclimatization

Hardening experiments were conducted for the study species by using various hardening media to determine the survivability rate of plantlets. For the node derived (88%) *in vitro* regenerated plantlets, the survivability rate was significantly higher in the hardening medium composed by garden soil, sand and vermicompost in the ratio of 1:1:1 by volume followed by the hardening medium consisting of red soil, sand and vermicompost in the ratio of 1:1:1 by volume for node derived (78%) *in vitro* regenerated plantlets (Table 3, Fig. 2i).

Table 3. Effect of different composition of hardening medium on survivability rate of node derived plantlets of the species, *Coscinium fenestratum.*

Hardening medium (v/v)	Number of plantlets transferred	Number of plantlets survived	Survivability (%)
Garden soil + sand + vermicompost (1:1:1)	50	44	88
Red soil + sand + vermicompost (1:1:1)	50	39	78
Vermicompost + red soil (1:1)	50	25	50
Red soil + sand (1:1)	50	22	44
Decomposed coir waste + perlite + vermicompost (1:1:1)	50	35	70

4. DISCUSSION

Generally, *in vitro* regeneration techniques have been found to be effective in circumventing cross ability barriers encounted in conventional methods. A wide range of Menispermaceae plants have now been successfully propagated using *in vitro* techniques (*Tinospora cordifolia* (7,8), *Tinospora formanii* (9) and *Cissampelos pareira* (10). This technique facilitates the introduction of successfully produced *in vitro* plantlets into the suitable micro-sites in natural communities to enhance the population of valuable plant species which have failed/less efficient in natural reproduction processes. The supplementation of growth hormones like auxins and cytokinins individually or in combinations with MS medium at different concentrations is having varied response with respect to callus formation and organogenesis

in many plant species (11). In the present study, the *in vitro* regeneration responses of the study species, *Coscinium fenestratum* are discussed below.

The subculturing of node for shoot formation of the species, *Coscinium fenestratum* onto the MS medium containing BAP (2.0mg/L) + TDZ (1.0mg/L) was found to be most effective. Cytokinins are one of the most important hormones for shoot proliferation in many plant species (12). Further, a wider survey of literature suggests that BAP is the most reliable and effective cytokinin (13). Kyozuka (14) reported that cytokinins can induce activation of meristems and cause shoot proliferation. Thomas and Gangaprasad (15) already reported the requirement of more quantity of BAP for effective shoot formation in the species, *Enicotema axillare.* Amin *et al.* (16) explained that in many cases, the growth regulators like many kinds of cytokinins served as better available source of nitrogen for organogenesis particularly shoot formation. Similarly, in *Vitex negundo,* Sahoo and Chand (17) reported BAP as the most effective growth hormone for shoot bud induction. Multiple shoots were formed from epicotyl explants of *Coscinium ferestratum* on MS medium supplemented with cytokinins (18,19). Huetteman and Preece (20) reported that Thidiazuron is a potent cytokinin hormone used for the growth of woody plants in tissue culture.

The root induction during the subculturing of node derived shoots was significantly higher in the half strength MS medium with IBA at 0.6mg/L. It indicates that the growth regulator, auxin is most essential for rooting attribute. Similar trend of results have been reported in other Menispermaceae member, *Tinospora cordifolia* (8). Tanimoto (21) have already reported the importance of auxins in the root formation during the subculturing of secondary explants. Similar kind of observation on the low level requirement of auxins for better rooting was reported in many species (22-24).

Hardening is a crucial step prior to transplantation of plant to soil. The well developed plantlets of the study species were acclimatized in various potting media. The node derived plantlets were responded well in the medium containing garden soil, sand and vermicompost. It may be due to the presence of suitable physical and chemical conditions of respective potting media for the survivability of these study species. The same hardening medium compositions were used by Jamuna and Paulsamy (25,26) for the growth of the medicinal plant, *Hypochaeris radicata.* These specific hardening media was also recommended for the micropropagation of epicotyl explants of the study species, *Coscinium fenestratum* for better survival in the field and they were morphologically similar to the mother plants (19).

From the present study, it can be concluded that the auxin and cytokinin interact synergistically to control the balance of cell division and differentiation. The protocol developed can be used to regenerate the species massively and hence for commercial purpose.

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RESEARCH ARTICLE

PREDATOPHILY- A NEW POLLINATION MECHANISM REPORTED IN WESTERN GHATS

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ABSTRACT

Predatophily – a new pollination mechanism has been described from *Impatiens*, *Sonerila* and *Strobilanthes* species in the Western Ghats of India.

Keywords: Predatophily, pollination, *Impatiens*, *Sonerila*, *Strobilanthes*, Western Ghats.

1. INTRODUCTION

The term pollination refers to transfer of pollen grains from anther to stigma. Pollen grains from most flowering plants are transported by various means and deposited on the receptive surface of the stigma of same flower or on the different flower of the same plant (self pollination) or on a different individual (cross-pollination). Forest vegetation with different flora and vegetation types harbor various assemblages of flower visitors, and thus the properties of plant – pollinator interactions are greatly affected by the composition of regional biota (1). Changes in pollinator regimes are thought to be of critical importance in speciation and diversification of the flowering plants. Such changes include qualitative shifts from one type of pollinator to another, quantitative shifts in the relative proportions of major types, and overall reductions in visits by animal pollinators that select for wind pollination or self fertilization. In the past few decades, biologists have made considerable progress in testing the role of pollinator shifts in fundamental aspects of the origin of species: diversification of floral traits and development of reproductive barriers between incipient species (2). They have also begun to evaluate the role played by recent changes in pollinator regime for the potential loss of species. In this context, study on the pollination mechanism of flowering plants and interaction of pollinators in reproductive biology of plants are most important. The present study observed the comparative pollination systems in three endemic flowering plants of Western Ghats. The study noticed a new pollination mechanism operated in the succession of plant life which has been described herewith.

2. MATERIALS AND METHODS

The pollination biology of *I. parasitica* carried out in about 50 flowering individuals growing in Munnar ranges of Western Ghats (N 10^{0}02.097'- E 77^{0}08.492', altitude 1300-1500 M msl) during 2009-2011. Whereas, observation of breeding systems in *Sonerila pulneyensis* were carried out from the Megamalai Wildlife Sanctuary (N 9^{0}40.769' – E 77^{0}24.119', altitude 1250-1350 M, msl) during 2012-2013. Floral visitors of *Strobilanthes kunthianus* were observed from the Megamalai ranges (N 9^{0}39.999' – E 77^{0}21.819', altitude 1150-1450 M, msl) during the month of August 2014. All the three plants observed breeding systems, floral traits, pollination patterns, floral visitors, and fruit setting frequency, ovule pollen ratio and seed setting frequency for studying reproductive success of the selected species (3). There has been observed a special pollen transfer mechanism by the non-nectar consuming agencies like toads and spiders which are acting as indirect pollen transfer agencies by the activity of predation. Hence it has been described here as a new pollination mechanism from the Western Ghats.

3. RESULTS AND DISCUSSION

Most species of *Impatiens* have conspicuous and specialized flowers providing a large amount of floral nectar and attracting nectar-feeding insects, bees, hawk moths_and butterflies for pollination (4); however, I have discovered a new mechanism of pollination in the flowers of an epiphytic perennial balsam (*Impatiens parasitica* Wight) which is restricted to southern Western Ghat ranges, in which the pollens are transported from the anther to stigma by the predatory activity of the toad (*Philautus jayarami*). Anthesis occurs (at 0750 to 0150) with mild odor and anthers dehisce at the same time. Flower secretes floral nectar in the base of inner petals and inflated spur (± 8-14µL/flower). The odor is released only up to noon, gradually getting weaker and none in night. The flowers usually wither up to 72 hours. The floral visitor (small tiny Dipterans, Hemipterans and Coleopterans) enters into the wide mouth of flower and touches their wings and head to anther and

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stigma. Another vertebrate pollinator (toad- *P. jayarami*) is waiting in front of the floral mouth for prey the floral visitors (Fig. 1). After foraging activity, flies return back to mouth of the flower. The toad is ready to catch the flies with its long rolling tongue. When catching the prey, tongue and nose of the toad touches the anthers and stigma of the flower and some amount of pollen carried on them. Sometimes tiny flies escape from the toads and starts foraging to next flower. Toad is also jumping to next flower to catch the fly and doing same activity several times for several flowers which transfer amount of pollen load and deposited on the other flower in the same plant or another individual. It leads a new mechanism of Predatophily, which is to the knowledge of science the first report of pollination of its own kind in angiosperms.

Fig. 1. *Philautus jayarami* – a toad is waiting for insects in front of the *Impatiens* flower.

Fig. 2. A tiny toad is predate on the flower petal of *Sonerila pulnyensis* Gamble.

Fig. 3. Spider predating inside the corolla tube of *Strobilanthes kunthianus.*

Similar observation was noticed in *Sonerila pulnyensis* Gamble, an endemic herb from the southern Western Ghats (Fig. 2). *S. pulnyensis* is blooming in the month of December; flower attracts several pollinators by their colour and pollen nectar. Many tiny insects are regular pollinators for this species. During this observation a small toad species frequently jumped on the flower petals and doing their rapacious activity. The same time pollen grains are dusted on the body of toads (12.5 ± 2.1 per visit) which transfer to another flowers stigma when continuing its predatory processes. This predatory mechanism leads to the transfer of pollen grains from one flower to another receptive part of the same individual or another individual. This is the very interesting and the first report of pollination mechanism in angiosperms for coining the term 'Predatophily'.

However in the tubular corolla of *Strobilanthes kunthianus* consists of many small spiders inside for predation of their prey as floral visitors (Fig. 3). While movement of spiders inside the corolla tube facilitates the pollen transfer to the other flowers stigma and its own. Considerable pollen load has been observed (10.2 ± 1.8 per visit) on the spider body which has been loaded on the receptive parts of the flower. *S. kunthianus* is a self-compatible floral species and also providing rich nectar resources to foraging agencies (3). Earlier studies it was not noticed the activity of non-nectar consuming agencies for pollination mechanisms. A few studies mentioned the nectar robberies which the small predating agencies act as a pseudopollinators in flowering plants.

4. CONCLUSION

All the above mentioned reports evidenced the new pollination mechanism operated in the succession of flowering plant reproduction. It has been reported first time the new pollination mechanism and has termed 'predatophily' due to

the activity of small animals such as toads and spiders.

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RESEARCH ARTICLE

ENVIRONMENTAL DEGRADATION AND HEALTH ISSUES

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ABSTRACT

Nature provides all kinds of facilities and resources to live in the planet. Nations are exploiting the environment (resources) as much as for comfort and luxury life in terms of development. The so called developments create negative impact in the planet and make the people keep away from nature. The environmental degradation is caused by combustion of fossil fuel, agricultural activities, industries, households, nuclear plants and other sources. These are polluting air, water and soil. As a result climate is changed and it leads global warming, flood, Hurricane, and other natural calamities. These incidences are led to threat to human health. The climate change leads to health problems such as malaria, dengue, yellow fever, diarrhoea, measles and other vector borne diseases, cancer, cardio vascular and respiratory diseases. The environmental degradation affects the food chain and it affects the health of the human beings. The climate change affects four grain production and it creates food insecurity. The poor people are forced to fall under malnutrition and it affects the health of the people. There is an urgent need to protect the environment and save the planet and protect the human beings from ill health.

Keywords: Health, pollution, need, climate change and urgent.

1. INTRODUCTION

Nature provides all kinds of facilities to live in the planet. For leading of life, all resources are supplied by environment. The environment is exploited as much as for our of comfort and luxury life. People enjoy the comfort and luxury life we termed as development. This development is without sustainability. The policy makers are thinking only the so called development rather than real development. This development can be achieved through industrialization. This industrialisation leads to environmental degradation. The industrialisation wastes are disposed freely into the environment. As a result environment is highly polluted by the industries. The high level of pollution is caused by too much exploitation of environment to meet the increasing demand for the people. The pollution is caused to climate change, ultimately there is a threat to the universe and health of the human beings throughout the world, climate change affects the human health at present and near future. There are reasons for climate change.

2. CARBON DIOXIDE CONCENTRATION

The climate change is caused by too much concentration of green house gases. These gases have been emitted from combustion of fossil fuel, forest fire, households, industries and agricultural activities. Among the green house gases, carbon dioxide plays a vital role to change climate. The major portion comes from combustion of fossil fuel. The high income countries energy related per capital emission CO_2 increased from 10.7 mt in 1990 to 11.1 mt in 2005. World per capital Co_2 emission increased from 4 mt in 1990 to 4.2 mt in 2005. The high income countries are damaging air more than low income countries in the name of development.

The high level of emission began from industrial revolution. The industrialisation has made unsustainable level of exploitation of natural resources. As a result the atmospheric Co_2 concentration increased from 280 ppm in 1760 to 379 ppm in 2000. If the trend continues, Co_2 concentration will increase to 560 ppm in the end of the century. The CH_4 and N_2O increased from 770 ppb to 1774 ppb and 270 ppb to 319 ppb respectively during the same period. The Co_2 concentration in the atmosphere doubles the pre-industrial levels by the end of the country. The other green house gases such as methane, black carbon and nitrous oxide contribute about 25 percent of the global warming.

3. IMPACT OF CO_2 CONCENTRATION

The impact of Co_2 concentration in the atmosphere leads to high temperature in the earth. This is termed as global warming. The global warming has been realised by all over the world. The global warming is resulted in negative impact on physical structure of the planet.

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4. RISING SEA LEVEL

Sea level has been changed due to ice melting of Arctic and Antarctic region. The past one century the sea level rose to 10 – 20 cm (4.8 inches). The Inter- Governmental Panel on climate change 2001 assessment projected that the sea level could rise as much as one meter in the end of the century. If the present global warming continues, one millimetre rise in sea level retreats and average of 1.5 meter seashore, one meter rise in sea level will retreat 1500 meter seashore in nearly a mile. This will create threat to coastal areas and islands our entire world.

5. HEAT WAVES

The global warming is resulted in heat waves. The global average surface temperature has increased and 100 years (1906 – 2005) indicated and increase of 0.74^{o}C + -01.8^{o}C. If the concentration of Co2 reaches 560 ppm, the temperature is projected to increase 1.4^{o}C to 5.8^{o}C.

Table 1. Temperature and Heat Waves.

Nations	Change in Temperature in ^{0}C 2000 - 2050	Change in heat wave duration (in days) 2000 – 2050
Algeria	1.9	22.2
Belarus	1.7	28.8
Finland	2.1	29.6
France	1.5	12.3
Czech Republic	1.7	20.3
Hungary	1.9	25.0
Kazakhstan	1.8	28.5
Poland	1.7	28.9
Romania	1.7	28.9
Russian Federation	2.2	29.5
Ukraine	1.7	28.5
USA	1.8	24.4
China	1.7	16.1
India	1.6	10.8
Canada	2.1	28.2
Ghana	1.3	1.3
Philippines	1.2	1.3
Togo	1.3	1.5

Source: World Development Report, 2010 (5)

Table 1 indicates that Finland will have 29.6 days in 2050 which is higher than all other countries. Majority of the countries will have more 10 days of heat wave in near future. This heat wave will increase mortality rate among children and old age people in the world.

6. NATURAL CALAMITIES

The natural calamities are caused by environmental degradation. They are flood, heavy rainfall, earthquake, cyclone, hurricane, volcano, tsunami, and unseasonal rainfall canoed by climate change. These affected the life, health and huge economic loss to the people. There were ten nations chosen to estimate the affected people.

Table 2. Natural Disasters and People Affected 1971 – 2008.

Nations	Drought No.	Flood & Strom of People	Share of Population
Bangladesh	658	8751	9.1
China	9642	53460	5.2
Ethiopia	1361	59	6.6
India	25294	22314	7.2
Pakistan	58	1163	1.3
Kaya	960	56	9.7
Philippines	172	2743	4.5
Sudan	611	155	6.0
Swaziland	43	23	18.3
Malawi	518	50	12.3

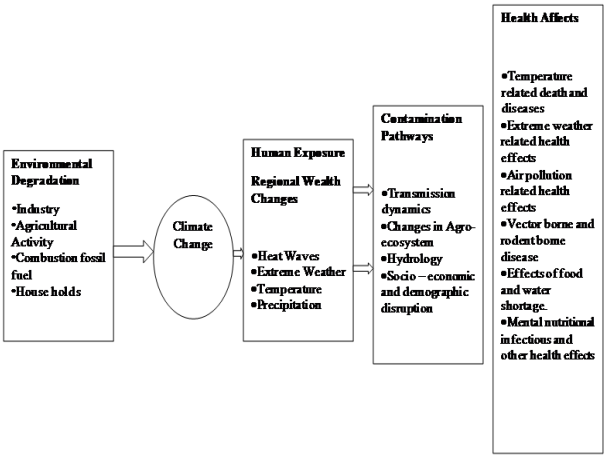
Source: World Development Report, 2010 (5)

Table 2 depicts that Bangladesh 9.1 percent, India 7.2 percent, Swaziland 18.3 percent and Malawi 12.3 percent of the total population were affected by drought and flood and storms.

7. CLIMATE CHANGE AND HEALTH ISSUES

Climate change is caused by environmental degradation. The impacts of climate change and variability on human health has increased considerably in recent years.

Environmental Degradation, Climate Change and Human Health



8. TEMPERATURE AND HEALTH

The hot temperature leads to heat wave. The heat wave creates spatial death and affects the health of human beings. This affects more children and old age people of the world. The recent heat wave was killed 70000 people in Europe. In India 18 heat waves have been reported in between 1980 – 1998. There were 1300 death in 1998 and 3000 death in 2003. The years 1998 and 2005 – 2007 were warmest on record. In many high temperature countries death rate during winter season is 10 – 25 percent higher than summer season. The high temperature leads to skin cancer. The excess deaths during times of thermal extreme are in person with pre-existing diseases especially cardio-vascular and respiratory diseases. The very old the very young and the frail are more susceptible.

9. EXTREME CLIMATE / WEATHER EVENTS AND DEATH

Extreme climate or weather kill people and affect the health of the people. On temperature countries undergoing climate change, a reduction in winter death may outnumber the increase in summer deaths.

Table 3. Extreme climate / weather events and death.

Nations	1990s		
	Events	Killed (Thousand)	Affected (Million)
Africa	24	10	104.3
Eastern Europe	150	5	12.4
Eastern Mediterranean	139	14	36.7
Latin America and Caribbean	298	59	36.7
South East Africa	286	458	427.4
Western Pacific	381	48	1199.8
Developed	577	6	40.8
Total	2078	601	1851

Source: WHO, Report 2010 (6)

Table 3 – indicates that the number of events increased from 1848 during 1980's to 2078 during 1990's similarly affected people increased from 1336 million to 1851 million during the same periods. This infers that number events have been increased and affected people increased due climate change.

10. CHILDREN'S HEALTH AND CLIMATE CHANGE

Children are highly affected both directly and indirectly. The World Health Organisation estimated that 34 percent of all childhood illness and 36 percent of children death under the age of 14 years are due to modifiable environmental factors in the world. Environmental changes can lead to respiratory diseases, sun burn, melanoma and immune – suppression. Climate change leads to heat strokes, gastro-intestinal diseases and psycho-social mal development. More number of children is affected by measles.

11. CLIMATE CHANGE AND MALARIA

Climate factors are an important determinant of various vector borne diseases. The increased temperature increases vectors life cycle and shortens the incubation time of parasites living the in the vector, climate change affects the reproduction and survival rates of both the infectious agents and vectors and therefore increase their ability to infect human. According to World Health Report 2002, approximately 6 percent of people affect by malaria in some middle income countries due to climate change. In tropical areas almost – 1 million people were dies a year (mostly children) and climate is projected to expose 90 million more people to the disease by 2030 in Africa alone.

Malaria causes 350 – 500 million illness per year and more than one million death mostly young children. Home grown malaria is caused by global warming (8).

Table 4. Climate Change and Malaria and Measles infected people.

WHO Region	2008	
	Malaria (in No)	Measles (in No)
African Region	607315	37010
Region of Americas	719783	203
South East Asian Region	100491743	75770
European Region	-	8883
Eastern Mediterranean Region	8291229	12120
Western Pacific Region	2604165	147986
Low Income	54504086	38174
Lower Middle Income	117031249	222431
Upper Middle Income	1395416	744
High Income Group	-	20623
Global	172997420	281972

Source: World Health Statistics, WHO, 2010 (1)

Table 4 shows that high level of incidence of malaria and measles were found in African and South East Asian region. The high level of infected persons was reported from lower middle income countries. If the temperature increases more the rate of malaria and measles infected patients will increase in near future. This will affect people and it leads to economic loss.

12. CLIMATE CHANGE AND DIARRHOEAL DISEASES

The diarrhoeal diseases are resulted in high temperature, water scarcity and water abundance from flooding or heavy precipitation. After a flood event rate of diarrhoeal diseases may increase. Even heavy rainfall increases the rate of diarrhoeal. Water scarcity may lead to diarrhoeal diseases. A shortage of availability of water for personal hygiene and washing food may lead to an increase in diarrhoeal diseases. High temperature is an independent risk factor of increased rate of diarrhoeal diseases. Diarrhoeal pathogens are highly sensitive to variation of climate and weather. Temperature and humidity have a direct influence on the rate of survival and replication of bacterial and protozoa. Paediatric hospitalisation for diarrhoea cases increased by 8 percent for 1oC increase in temperature in developing countries, diarrhoea incidence will increase by 5 percent per degree Celsius increase in temperature. The burden of diarrhoeal diseases from climate change is projected to increase up to 5 percent by 2020 in countries with per capital income below $6000.

13. CLIMATE CHANGE AND DENGUE

Dengue is a climate and weather sensitive disease. This is an important arboreal disease of human beings, occurring in tropical and sub-tropical regions. EI Nino Southern Oscillation affects dengue occurrence by causing changes in household water pooling. The number dengue infected has increased dramatically in the past 30 years. There were 1.2 million cases reported from 56 countries in 2000. Dengue has been expanding its geographic range and climate change is expected to double the rate of people at risk from 30 percent to up to 60 percent of the world population (or 5 billion to 6 billion by 2070). It is estimated that each year 50 million infectious occur with 500000 cases of dengue fever and at least death mainly among children.

14. AIR QUALITY AND HEALTH

Air quality is damaged by industrial wastes, emission from automobiles, agricultural activities and indoor pollution. Air pollution is determined in part by climate factors such as temperature and humidity. The transport and dispersion of air pollutants away from source regions are highly affected by weather factors. Climate change is weather factors. Climate change is influenced by air quality which is turned to affect the health of people. Air pollution related diseases are cardio vascular and respiratory diseases. Exposure to high levels of ground level ozone is formed from the exhaust of transport vehicles. This increases the risk of exacerbations of respiratory diseases such as chronic obstructive airways disease and asthma leading to hospital admission or increased mortality. Cardio – vascular diseases is a leaching death in the United States. In 2007 of all Americans who died of Cardio-vascular diseases 1, 50,000 were younger than age 65.

15. CLIMATE CHANGE AND MALNUTRITION

The climate change affects agriculture directly. The unseasonal rainfall and hot temperature create shortage of water. Stalinisation of agricultural land due to rise sea level and it decreases yield. Flood events, heavy rainfall, drought and shortage of water affect the harvest of food grains. There create food grain shortage for consumption. This situation will create food insecurity. The food insecurity leads to malnutrition. The immune is weaker while a person suffering from malnutrition. The vector born diseases are affecting more among them. The incidence of ill health; will increase in near future.

FAO estimated that 850 million people were undernourished in 2007. Climate change is expected to increase the number of undernourished people between 35 and 170 million in 2080.

Table 5. Undernourished population in Africa and developing world.

Region	Total Undernourished
North Africa	61,000,000
West Africa	34,400,000
South Africa	35,700,000
East Africa	92,400,000
Central Africa	45,200,000
Developing World	797,900,000

Source: UNDP Report, 2007 – 09 (7)

Table 5 depicts that more number of people in Africa and developing world living with undernourished. This population faces the problem of ill-health's more than the counterpart. The probable chance of incidence of disease is higher for them.

16. CONCLUSION

The environmental degradation leads to climate change all over the world. Climate change creates negative impact on physically and psycho social economic conditions of people in the world. Hot temperature increased the life cycle of vectors. This leads to increase the rate of malaria, dengue, yellow fever, diarrhoea and other related diseases. Similarly increases the mortality among pre-affected diseases of cardio vascular and respiratory. These kinds of health issues are due to so called development. We need development but without harmless to environment and human beings. The need for an hour is to mitigate the pollution, save environment and create awareness among people to adopt with changes. The healthy people can build strong nation. Health is wealth that must be protected.

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RESEARCH ARTICLE

ASSOCIATION OF ARBUSCULAR MYCORRHIZAL FUNGAL SPECIES IN THE PLANT SPECIES OF BARGUR HILLS, ERODE DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

The present study was carried out the arbuscular mycorrhizal fungal root colonization and spore population diversity some medicinal plants species at Bargur hills Western Ghats of (Anthyur taluk), Erode district, Tamil Nadu, India. Root and rhizosphere soil samples were collected during the month of August, 2017-March, 2018 from the surface to 20 cm depth as well as pH were also measured. Totally 25 plant species belonging to 19 families recovered Arbuscular mycorrhizal fungal spore and root colonization. The results of the present study arbuscular mycorrhizal spore population in the rhizosphere soil and root colonization of all the plant species. A total of 22 AM fungal species belonging to 7 genera and 2 different orders were recorded from the rhizosphere soil samples of this study region. The *Glomus* was dominant had seen in rhizosphere soil samples in all the medicinal plant species. The maximum spore population was found in the rhizosphere soil samples of *Leucas aspera* (470 /100 g soil) which belongs to the family Lamiaceae and lowest spore population was observed in the *Tephorosia purpurea* (123 /100g soil) belongs to Fabaceae. The highest 83 % AM fungal infection was found in roots of *Achyranthus aspera* belongs to the family Amaranthaceae, while the lowest 23 % AM fungal association was found in the root of *Mimosa pudica* belongs to the family Mimosaceae.

Keywords: Arbuscular mycorrhizal fungi**,** Bargur hills.

1. INTRODUCTION

Forest plays an essential role in maintaining the environmental and bio resources stability and provides multipurpose benefit to the mankind. Mycorrhizal association is essential for forest trees. AMF diversity may equip both tree and forest to functionally adapt to changes in seasons and habitats. Arbuscular Mycorrhizal fungi from symbiotic association with about 90% of the families of all phyla of land plants (1) including ferns and some mosses (2). In Arbuscular Mycorrhizal fungal symbiosis in rhizosphere soils a dynamic process and interaction effects all physiological aspects of the plant host. This fungus have a great potential to enhance plant growth by increase uptake of nutrients especially in phosphorus (3). A major beneficial component of soil microbial community is mycorrhizal fungus, which contributes to plant growth and survival by reducing stresses through symbiosis (4). The mycorrhizal are very common in disturbed areas which indicate their positive role in establishing and building the plant community. The mycorrhizal associations are essential to the colonization of nutrient -deficient soils.

Even the modest things are vital to the world particularly in relation to getting plants established. The mycorrhizal fungi inhabit plant roots and extend the root system into the adjoining soil. Unexpected quantities of mycorrhizal filaments are found available in healthy soil. An extremely small section of soil associated with dynamically growing plants may be full of numerous fungal filaments. The affiliation is favorable for the reason that the plants have the benefit of improved uptake of water and mineral nutrient, resistance against diseases, greater survival, and enhanced growth. Hence in this present study area of Bargur hills, there is no report of AM fungal spore population and root colonization in this study area.

2. MATERIALS AND METHODS

2.1. Study area-description

The present study was undertaken in the Bargur hills, Erode district of Tamil Nadu. Bargur hills are located at 77°20"60' E longitude and 11°37"60' N latitude and 950 m. s. l. above the sea level (Fig-1) Borders of Tamil Nadu and Karnataka regions and the continuation of Sathyamangalam forests, a mixed deciduous vegetation cover of Southern Eastern Ghats. A majority of primitive tribes Lingayath are inhibited in Thamarakarai village in Bargur hills. These hills have three major forest types are present with includes dry deciduous forest, moist deciduous and small patch of grasslands. The climates are mostly in moist

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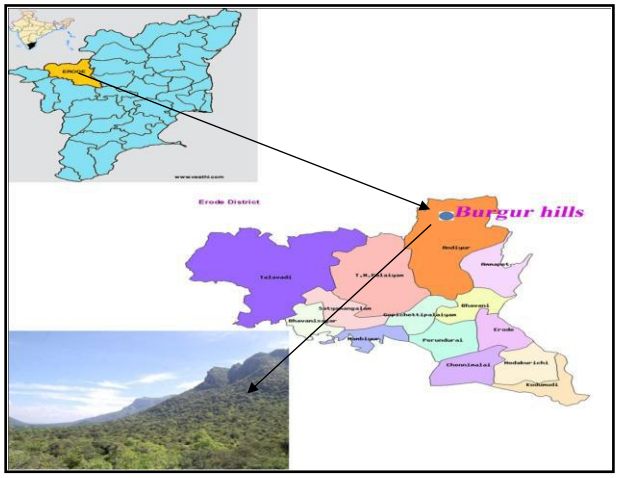
conditions and average rainfall ranging from 50 to 75 cm per year. Hence, the maximum temperature in 40°C to 45°C from April-May and minimum temperature in between 20°C to 25°C from November-December. The major irrigation sources of Palar River to provide water facilities from drinking and agriculture.



2.2. Sample collection

Present study the root and rhizosphere soils samples were collected from 25 plant species during the year August, 2017 to March, 2018. All the samples were placed in the olyethylene bags, labeled and then transported to the laboratory. The root samples were freshly processed, whereas rhizosphere soil samples were analyzed for mycorrhizal spore population and AM fungal root colonization.

2.3. Estimation of AM fungal root colonization

The fresh root samples were cleared and stained in tryphan blue following method of (5). Root samples of each plant species were washed gently under tap water and cleared in 2.5% KOH, acidified in 5 N HCL and stained in lacto glycerol with 0.05% Trypan blue. The stained roots were examined under a compound microscope (40x–100x). Hundred root segments for each sample were randomly selected for microscopic observation and the degree of colonization was estimated using the slide method.

The percentage of AM fungal infect ion was calculated using the formula:

$$\text{Percentage of infection} = \frac{\text{No. of root segments infected}}{\text{Total no of root segments observed}} \times 100$$

2.4. AMF spore identification

AM fungal spores were extracted from 100 g rhizosphere soil by wet-sieving and decanting method (6) through a series of 710 to 37µm size sieve filter. For the identification and nomenclature of these AM fungal spore synoptic keys developed by (7-9) were used. The classification was based upon the color, shape, hyphae, structure, size, and cell wall thickness and spore diameter.

2.5. Soil pH

The pH of the rhizosphere soil samples was determined (soil-water suspensions 1:5) with the help of pH meter (Elico) and values were recorded.

3. RESULTS AND DISCUSSION

The present study arbuscular mycorrhizal spore population and root colonization of totally 25 plant species belong to 19 families (Fig-2) and pH also measured. The maximum Arbuscular mycorrhizal fungal colonization was found in the roots of *Achyranthus aspera* (83%) belongs to Amaranthaceae and lower root colonization was found in *Mimosa pudica* (22%) belongs to the family Mimosaceae. The plant species like *cardiospermum luridum* (39%) belongs to Sapindaceae, *Hemidesmus indicus* (33%), (Apocynaceae), *Hibiscus micranthus* 30% (Malvaceae), *Commelina benghalensis* 24% (Commelinaceae), *Mimosa pudica* 22% (Mimosaceae) showed to 20 to 40% of infection. The plant species like *Abrus precatoris* (55%) (Fabaceae), *Barleria tomentosa* (44%) Acanthaceae, *Crotolaria pallida* 49% (Fabaceae), *Datura metal* 43% (Solanaceae), *pavonoia odorata* 60%; Malvaceae, *polygala javana* 53% (Polygalaceae), *Stachyrarpeta jamecensis* 57% (Verbinaceae), *Tephrosia purpurea* 46% (Fabaceae) showed to 60% of infection (Table-1; Fig-3, 4).

The other plant species *Barleria prionits* (72%) Acanthaceae, *Clitoria teranceae* (67%) (Fabaceae), *Canscorra decussate* (65%) (Gentinaceae), *Corchorus olitorius* 74%, (Tiliaceae), *Leuca saspra* (70%) Lamiaceae, *Strobilanthus clorota* (77%) Acanthaceae, *Strobilanthus consanguine* 68% (Acanthaceae), *Urena lobata* 63% (Malvaceae) showed 60 to 80% of infection. The one species belongs to Acanthaceae member *Achyranthus aspera* showed 83% of infection. This finding is anagreement with Miller (10) and D' Souza and Rodrigues (11). Variation in Arbuscular Mycorrhizal fungal association and spore number are known to be affected by rapid changes in soil nutrients, environmental factors, soil fertility or soil

disturbances in the sites. Majority of the belonging to the family Aizoaceae, Commelinaceae and Nyctaginaceae believed to be non-mycorrhizal plants were found to be associated with AM fungi. In the present study the commlinaceae member *Commeliana benghalensis* associated with AM fungi. The root of the *Com. benghalensis* showed 24% of infection. In the present study, the plant species belonging to non-mycotrophic families were found to be mycotrophic. This is accordance with Normal *et al*., (1995) who also indicated that some of the representatives of non-mycotrophic families viz. Amaranthaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Cyperaceae and Juncaceae could also form mycorrhizal association. In the present investigation the *Achranthus aspera* belongs to Amaranthaceae showed mycorrhizal infection. The present findings no correlation between mycorrhizal variables such as percentage of root length colonized by Arbuscular Mycorrhizal fungi, intensity of infection and spore density. The relationship between spore number, percentage colonization by AM fungi and intensity of infection is complicated as it is influenced by many environmental and biological factors.

In this study the rhizosphere soils sample of Bargur hills, totally 22 Arbuscular Mycorrhizal fungal species isolated and identified (Table-2; Fig-5, 6). Of these 1 species of *Ambispora*, *A. appendiculatum*, 2 species of *Funneliformis*, *F. fragilistratum*, *F. geosporum,* 14 species of *Glomus*, *G. heterosporum, G. hoi, G. invermeyanum, G. macroporum, G. maculosum, G. microsporum, G. magnicule, G. monosporum, G. multicaulis, G. multisubstensum, G. panishalos, G. radiatum, G. segmantatum, G. versifome*, 1 species of *Sclerocytes, S. pachycaulis,* 1 species of *Paraglomus*, *P.occultum,* 2 species of *Rhizophagus*, *R. intraradix, R.manihotis,* and, 1 species of *Pasiphora, P. dominika* recovered from the rhizosphere soil samples.

Fig. 2. Identification of some plant species in Bargur hills

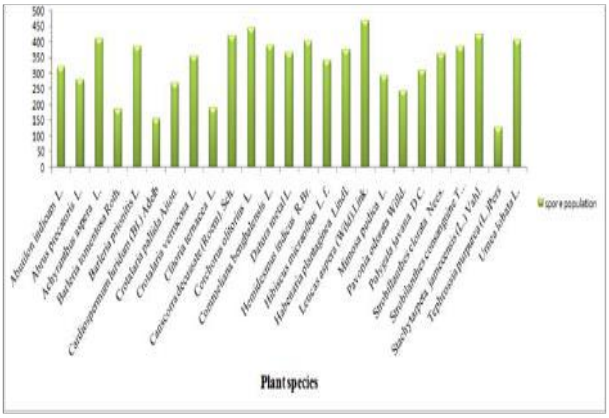


Fig. 3. AM fungal Spore population in rhizosphere soil samples of Bargur hills.

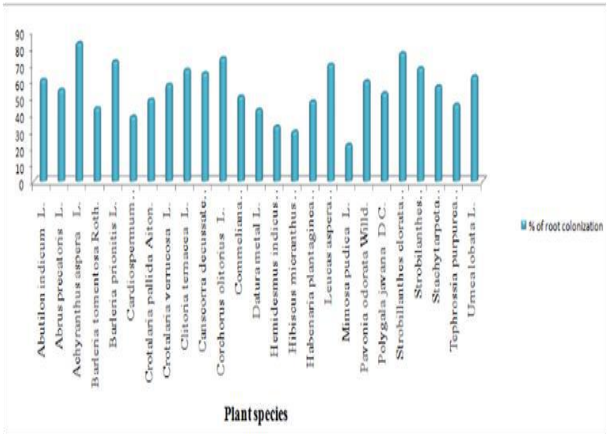


Fig. 4. AM fungal colonization in the root samples of Study area.

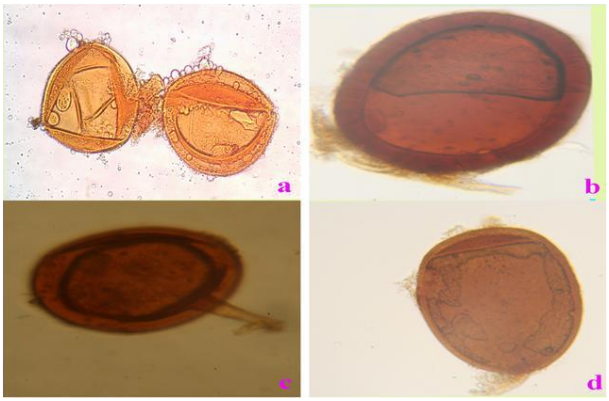


Fig. 5. AM fungal spores isolated from the rhizosphere soil of Bargur hills..

Similarly, Santhoshkumar and Nagarajan (12) studied in AM fungal spore population and root colonization in 20 plant species belonging to 20 families and they isolated from the rhizosphere soil totally 39 AM fungal species belongs to 6 genera identified.

Table 1. Arbuscular Mycorrhizal fungal spore population and root colonization in the plant species of Bargur hills, Anthiyur (Taulk), Erode district, Tamil Nadu, during 2017-2018.

S. No	Plant Species	pH	Types of infection			Spore Population (100g/soil)	(%) root colonization
			Hyphae	Arbuscule	Vesicles		
1.	*Abutilon indicum* L.	5.2	+	-	+	320	61
2.	*Abrus precatoris* L.	4.9	+	+	-	280	55
3.	*Achyranthus aspera* L.	6.2	+	+	-	410	83
4.	*Barleria tomentosa* Roth.	5.5	+	-	+	185	44
5.	*Barleria prionitis* L.	5.7	+	+	-	387	72
6.	*Cardiospermum luridum* (BI.) Adelb	6.0	+	-	+	156	39
7.	*Crotalaria pallida* Aiton.	4.8	+	+	-	270	49
8.	*Crotalaria verrucosa* L.	5.1	+	-	+	355	58
9.	*Clitoria ternacea* L.	4.9	+	+	-	190	67
10.	*Canscorra decussate* (Roem). Sch.	5.3	+	-	+	420	65
11.	*Corchorus olitorius* L.	6.7	+	+	-	445	74
12.	*Commeliana benghalensis* L.	6.6	+	-	+	390	51
13.	*Datura metal* L.	5.9	+	+	-	365	43
14.	*Hemidesmus indicus* R.Br.	5.4	+	-	+	402	33
15.	*Hibiscus micranthus* L. f.	5.3	+	-	+	339	30
16.	*Habenaria plantaginea* Lindl.	6.1	+	+	-	375	48
17.	*Leucas aspera* (Wild).Link.	6.4	+	-	+	470	70
18.	*Mimosa pudica* L.	5.2	+	+	-	293	22
19.	*Pavonia odorata* Wild.	6.3	+	-	+	245	60
20.	*Polygala javana* D C.	5.5	+	+	-	310	53
21.	*Strobillanthes clorata* Nees.	6.8	+	-	+	360	77
22.	*Strobilanthes consanguine* T. Anderson	5.7	+	-	+	387	68
23.	*Stachytarpeta jamecensis* (L.) Vahl.	6.9	+	-	+	422	57
24.	*Tephrossia purpurea* (L.)Pers	5.6	+	+	-	126	46
25.	*Urnea lobata* L.	6.1	+	-	+	405	63

Table 2. AM fungal genera and species were isolated from the rhizosphere soil samples in Bargur hills, Anthiyur, Erode district.

S.No.	AM fungal genera	AM fungal Species
1	*Ambispora*	*A. appendiculatum*
2	*Paraglomus*	*P.occultum*
3	*Pasipora*	*P. dominika*
4	*Glomus*	*G. heterosporum, G. hoi, G. invermeyanum, G.macroporum, Gl. maculosum, G. microsporum, G. magnicule, Gl. monosporum, G. multicaulis, G. multisubstensum, G.panishalos, G. radiatum, G. Segmantatum, G. versifome*,
5	*Rhizophagus*	*R. intraradix, R.manihotis*
6	*Funneliformis*	*F. fragilistratum*, *F. geosporum*
7	*Sclerocystis*	*S. pachycaulis*

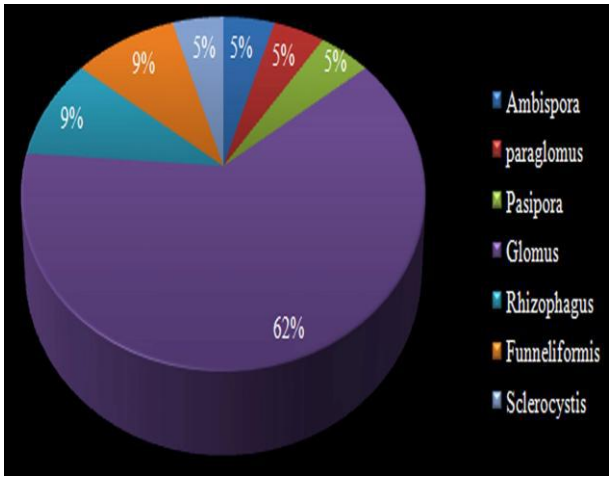


Fig. 6. Dominant Arbuscular Mycorrhizal fungal spores in rhizosphere soils samples of Bargur hills

4. CONCLUSION

In conclusion the present study revealed that the collected plant species from Bargur hills had rich population Arbuscular Mycorrhizal fungal spores and root colonization occurred in plant species. In this symbiotic association of AM fungi to absorb the soil nutrients, zinc, copper especially phosphorous and also increased plant growth biomass, diseases resistance and tolerence.

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RESEARCH ARTICLE

INHIBITION OF NITRIC OXIDE PRODUCTION IN LPS-STIMULATED RAW 264.7 CELLS BY *DOLICHANDRONE ATROVIRENS* BARK

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ABSTRACT

In traditional systems of medicine, the bark of *Dolichandroneatrovirens* has been used to treat various disorders. The main aim of this study was to determine the anti-inflammatory effect of *D. atrovirens* bark through the inhibition of nitric oxide (NO) production in lipopolysaccharide-stimulated RAW 264.7 cells. For this purpose, preliminary phytochemical composition and *in vitro* antioxidant activities of various solvent extracts of *D. atrovirens* bark were evaluated to select the most effective extract.The methanol extract of *D. atrovirens* registered the highest amount of total phenolics (476.2 mg GAE/g) and flavonoid (129.0 mg RE/g) contents witha strong antioxidant activity as measured in DPPH (IC_{50} of 19.52 µg/mL) and ABTS (IC_{50} of 10.82 µg/mL) scavenging activities.Hence, the methanol extract was selected for cell line study. Further, the methanol extract of *D. atrovirens* effectively inhibited the production of NO in RAW 264.7 cells induced by LPS (13.1 µM at the concentration of 80 µg/mL). It could be concluded that the presence of higher level of total phenolic components in the methanol extract of *D. atrovirens* bark might be responsible for reducing the NO level in cells.

Keywords: Antioxidant, *D. atrovirens*, Nitric oxide, lipopolysaccharide, RAW 264.7 cells.

1. INTRODUCTION

In the last few decades, numerous studies have been documented for the utilization of natural antioxidants as potential disease preventing agents to reduce the risk of cardiovascular diseases, neuro-degenerative diseases, inflammations, diabetes and cancers (1,2). The protective effects of the plants are mostly related to the antioxidant components such as phenolics, carotenoids, phytates, isothiocyanates, phytosterols, phytoesterogens and organosulfur (3). Hence, the search continues for the novel and effective antioxidants from the plant source to reduce the risk of free radical mediated disorders. Inflammation is the normal response of a living tissue to injury caused by physical or noxious chemical stimuli or microbiological toxins. Macrophages are the main pro-inflammatory cells responsible for invading pathogens by releasing many pro-inflammatory molecules such as nitric oxide (NO), prostaglandin E2 (PGE2), and of cytokines, like interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α)(4).

Among these, NO is an important molecule for host defense response against various pathogens such as bacteria, viruses, fungi, and parasites (5). Under normal physiological conditions, NO plays an important role in the regulation of various pathophysiological processes such as neuronal communication, vasodilatation, and neurotoxicity (6).However, overproduction of NO has been concerned in the development of various inflammatory diseases, such as arthritis, asthma, multiple sclerosis, inflammatory bowel disease, and atherosclerosis (7). Accordingly, the regulation of these pro-inflammatory mediators in lipopolysaccharide (LPS)-stimulated macrophage cell line is an effective therapeutic strategy for the development of novel anti-inflammatory agents.

Plants are important source of therapeutic drugs and play a significant role in the survival of the tribal and ethnic communities. Plants products are known to possess a variety of secondary metabolites which have various biological activities (8). A number of Indian plants have been investigated for their beneficial use as antioxidants using presently available experimental techniques. Recently, several experimental studies have contributed scientific evidence for the pharmacological effects of various medicinal plants observed in folk medicine. During the ethnobotanical interview, the traditional healers of Melur of Bodha hills are using the bark of *Dolichandroneatrovirens* (Roth) Sprague (Family: Bignoniaceae) to successfully treat inflammations. But there is no information available on pharmacological evaluation of *D. atrovirens.* Hence, the stem bark of *D. atrovirens*was rightly chosen in the present study for understanding its anti-inflammatory effect. Based on the above knowledge, the present investigation was

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undertaken to evaluate the antioxidant and anti-inflammatory (through the inhibition of NO production in RAW 264.7 cells activated with lipopolysaccharide) of *D. atrovirens* bark.

2. MATERIALS AND METHODS

2.1. Preparation of extracts

The bark sample of *D. atrovirens* was collected from Bodha hills, Melur, Southern Eastern Ghats, Tamil Nadu, India. The freshly collected bark samplewas washed thoroughly in tap water, shade dried at room temperature (25^{o}C), powdered, and used for solvent extraction. The plant materialwas successively extracted with n-hexane, chloroform, ethyl acetate and methanol using soxhlet apparatus and the air dried residues were further extracted with hot water by the method of maceration for 24 h. Each time before extracting with the next solvent, the material was dried in hot air oven at 40ºC. The solvents were evaporated using a rotary vacuum-evaporator and air dried. The extract recovery in different solvents was expressed as percent of the plant sample dry matter..

2.2. Determination of total phenolic, tannin, and flavonoid contents

The total phenolic contents of the bark were determined by FolinCiocalteu method. The amount of total phenolics was calculated as gallic acid equivalents (GAE) as described by Siddhuraju and Becker (9). The total flavonoid content was determined by the method described previously by Zhishen et al. (10) and expressed as gram of rutin equivalent (RE)/100 g of extract.

2.3.In vitro antioxidant activity

2.3.1. Antioxidant activity by ABTS$^{\bullet+}$and DPPH assays

The total antioxidant activity of the samples was measured by ABTS radical cation decolorization assay according to the method of Re *et al*. (11). The DPPH radical scavenging activity of different extracts of *D. atrovirens* bark was measured according to the method of Blois (12). IC_{50} values of the extract i.e., concentration of the extract necessary to decrease the initial concentration of DPPH or ABTS by 50% was calculated.

2.4. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells

Based on the results of antioxidant and cytotoxicity studies, methanol extract of bark was selected for further studies.

2.4.1. Cell culture

The murine macrophage RAW 264.7 cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in DMEM supplemented with 10% FBS, 100 µg/L streptomycin, and 100 IU/mL penicillin at 37ºC in a 5% CO_2 atmosphere (HERAcell 150, Thermo Electron Corp. Waltham, MA, USA).

2.4.2. Cell viability assay

The mitochondrial-dependent reduction of MTT to formazan was used to measure cell respiration as an indicator of cell viability. Briefly, RAW 264.7 cells were seeded in 96-well plates at the density of 5×10^4 cells/well. After 24 h of incubation, the adhered cells were treated with various concentrations of the extracts. Twenty four hours later, after changing the medium, MTT was added to a final concentration of 0.5 mg/mL, and the cells were incubated for 4 h at 37ºC and 5% CO_2. The medium was then removed and the formazan precipitate was solubilized in DMSO. The absorbance was measured at 550 nm on a microplate reader (Biotek, Winooski, VT, USA).

2.4.3. Inhibition of nitric oxide production

The RAW 264.7 cells were seeded at a density of 5×10^5 cells/well in 24 well plates and incubated for 12 h at 37ºC and 5% CO_2. Then media of each well were aspirated and fresh FBS-free DMEM media were replaced. Different concentrations of *D. atrovirens* extract were prepared in FBS-free DMEM to give a total volume of 500 µL in each well of a microtiter plate. After 1 h treatment, cells were stimulated with 1 µg/mL of LPS for 24 h (13).

The presence of nitrite was determined in cell culture media using commercial nitric oxide detection kit. Protocols supplied with assay kit used for the application of assay procedure. Briefly, 100 µL of cell culture medium with an equal volume of Griess reagent in a 96-well plate was incubated at room temperature for 10 min. Then the absorbance was measured at 540 nm in a microplate reader (Biotek, Winooski, VT, USA). The amount of nitrite in the media was calculated from sodium nitrite ($NaNO_2$) standard curve.

2.5. Statistical analysis

The values expressed are means of three replicate determinations ± standard deviation. The statistical analysis was carried out by analysis of variance (ANOVA) followed by Tukey's test. The data were evaluated with SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTSAND DISCUSSION

3.1. Preliminary phytochemical studies of different extracts of D. atrovirens stem bark

The yield percent, total phenolic, and flavonoid contents of the extracts obtained from *D. atrovirens* bark powder using hexane, chloroform, ethyl acetate, methanol and water are presented in Table 1. The maximum extract yield was obtained in the hot water extract (13.2%) followed by methanol extract (11.3%). The results of total phenolic and tannin contents are expressed as gallic acid equialents, whereas the flavonoid content is expressed as rutin equivalent. The extractable total phenolics (476.2 mg GAE/g extract) and flavonoids (129.0 mg RE/g extract) were found to be higher in the methanol extract of *D. atrovirens* bark. On the other hand, among the different sample extracts, the lowest concentrations of phenolics (65.7 mg GAE/g extract) and flavonoids (18.2 mg RE/g extract) were observed in the hexane extract.

Table 1. Extraction yield and total phenolic, tannin and flavonoid contents of different extracts of *D. atrovirens* bark.

Sample	Extract yield (%)	Total phenolics (mg GAE/g extract)	Flavonoid (mg RE/ g extract)
Hexane	0.2	65.7 ± 8.9^{e}	18.2 ± 0.8^{e}
Chloroform	0.8	127.3 ± 11.8^{d}	48.6 ± 1.9^{d}
Ethyl acetate	1.7	361.4 ± 20.2^{c}	87.2 ± 2.7^{c}
Methanol	11.3	476.2 ± 19.1^{a}	129.0 ± 3.7^{a}
Water	13.2	398.8 ± 18.7^{b}	97.6 ± 2.6^{b}

Total phenolic content is expressed as gallic acid equivalent (GAE)
Flavonoid content is expressed as rutin equivalent (RE).
Values are mean of three replicate determinations (n =3) ± standard deviation.
Mean values followed by different superscripts in a column are significantly different (P< 0.05).

Plant phenolics have received considerable attention because of their potential biological activity. Phenolic compounds such as flavonoids, phenolics acid, and tannins possess diverse biological activities including anti-inflammatory, anti-carcinogenic, and antiatherosclerotic activities. These activities might be related to their antioxidant activity (14). The higher amount of phenolics in the methanol extract of *D. atrovirens*could be due to higher solubility of phenolic compounds. Phenolics are powerful antioxidants and act in a structure-dependent manner; they can scavenge reactive oxygen species, and chelate transition metals which play vital roles in the initiation of deleterious free radical reactions (15). Obviously, total phenolic content could be regarded as an important indication of antioxidant properties of plant extracts. Crude extracts of fruits, vegetables, and other plant materials are rich in phenolics(16).Since the extracts of *D. atrovirens* bark possess appreciable phenolic, and flavonoid contents, it can be taken as a good indication for its higher antioxidant capacity. There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidant activity of these compounds (17).

3.2. In vitro antioxidant assays

3.2.1. Free radical scavenging activity on DPPH

The scavenging abilities of different solvent extracts of *D. atrovirens* bark were concentration-dependent and expressed as IC_{50} values (Figure 1). Concentration of the sample necessary to decrease the initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was calculated. All the extracts exhibited appreciable DPPH radical scavenging activity ranging from IC_{50} 19.52 µg/mL (methanol extract) to IC_{50} 241.99 µg/mL (hexane extract). DPPH free radical scavenging effect of *D. atrovirens* bark extracts and quercetin was in this order: Quercetin>methanol>water >ethyl acetate > chloroform>hexane.

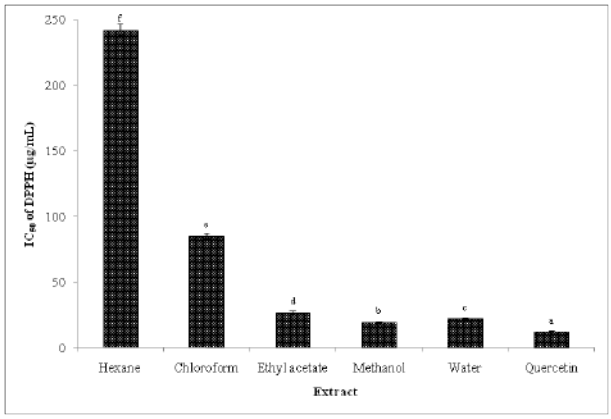

Fig. 1. DPPH radical scavenging activity of different extracts of *D. atrovirens* bark.

The DPPH is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by antioxidant. DPPH scavenging assay is routinely practiced for assessment of free radical scavenging potential of an antioxidant molecule and considered as one of the standard and easy colorimetric methods for the evaluation of antioxidant properties of pure compounds (18). The principle of this assay is

DPPH• on accepting a hydrogen (H) atom from the scavenger molecule i.e. antioxidants; the purple color of the DPPH thus changes to yellow which indicates that scavenging reaction (19). The antiradical scavenging activities of different extracts of *D. atrovirens* bark were also in agreement with the above reports and would be related to the nature of phenolics, thus contributing to their electron transfer/ hydrogen donating ability (Figure 1). Antioxidants with DPPH radical scavenging activity could donate hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major propagators of the chain autoxidation of lipids, and to form nonradical species, resulting in the inhibition of propagating phase of lipid peroxidation (20). Previous literatures have also expressed the positive correlation between the polyphenolic compounds and DPPH radical scavenging activity (21,22).

3.2.2. Antioxidant activity by the ABTS•+ assay

The different extracts from the *D. atrovirens* bark were fast and effective scavengers of the ABTS radical (Figure 2). In ABTS•+ scavenging activity, the IC_{50} values varied significantly ($P < 0.05$) and ranged from 10.82 to 128.6 µg/mL. Similar to DPPH radical scavenging activity, methanol extract showed the highest ABTS radical scavenging activity than other extracts (IC_{50}at 10.82 µg/mL). The lowest ABTS radical scavenging activity was found in the hexane extract with the IC_{50} value of 128.6 µg/mL.

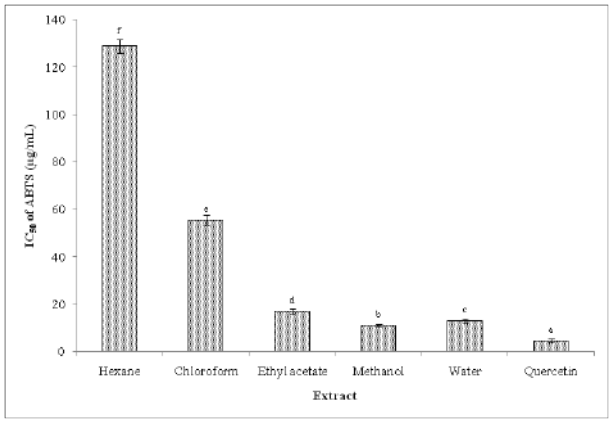

Fig. 2. ABTS radical scavenging activity of different extracts of *D. atrovirens* bark.

Scavenging of ABTS•+ is a simple and inexpensive method used to evaluate the radical scavenging ability of the plant extracts. Generation of ABTS•+ is from oxidation of ABTS by potassium persulfate and determines the antioxidant activity of electron donating antioxidants (scavengers of aqueous phase radicals) and chain breaking antioxidants (scavengers of lipid peroxyl radicals) (23). ABTS•+ also involves an electron transfer process. In the present study, the obtained results clearly indicate that all the tested extracts effectively inhibited and scavenged the radicals. Actually, the ABTS radical cation scavenging activity also reflects hydrogen-donating ability. The high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS•+) (24). Since, the extracts from *D. atrovirens* bark sample have the ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction, they could serve as potential nutraceuticals when ingested along with nutrient.

The results of *in vitro* antioxidant tests have shown that the methanol extract of *D. atrovirens* as good antioxidant and free radical scavenger. The highest amount of total phenolics and antioxidant activity was shown by the methanol extract of *D. atrovirens*. So this extract was chosen for the further investigations.

3.4. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells

In the present study, methanol extract of *D. atrovirens* bark was evaluated for the inhibition of NO production in the LPS-stimulated RAW264.7 cells. The nitrite accumulation in the cells increased due to the LPS treatment. When compared to the untreated control, the pre-treated cells induced with LPS released a lower level of NO in the medium measured as its stable non-volatile breakdown product nitrite. The methanol extract remarkably inhibited ($P< 0.05$) the nitrite accumulation in LPS-stimulated RAW 264.7 cells in a concentration dependent manner. The methanol extract exhibited the reduction of nitrite level to 13.1 µM at the concentration of 80 µg/mL. The result of MTT cell viability assay revealed that the inhibitory effect of methanol extract was not due to cell damage (viability >90%) (Figures 3and 4).

Macrophages play important roles in inflammation through the production of several pro-inflammatory molecules, including NO. Production of excessive NO has been associated with a range of inflammatory diseases including arteriosclerosis, ischemic reperfusion, hypertension and septic shock (25,26). Recent studies have demonstrated that the plant foods including fruits, vegetables and medicinal herbs are an excellent source of antioxidant molecules that effectively inhibit the inflammatory process by affecting different molecular targets (27,28). In this study, methanol extract of *D. atrovirens* bark exhibited a higher level of total phenolics when compared with other extracts. In addition, phenolic

compounds are known to be potent for inhibiting NO and peroxynitrite productions (29). Higher level of polyphenol content with strong antioxidant potential of pant samples are a good target for examining the inhibitory activity against NO production.

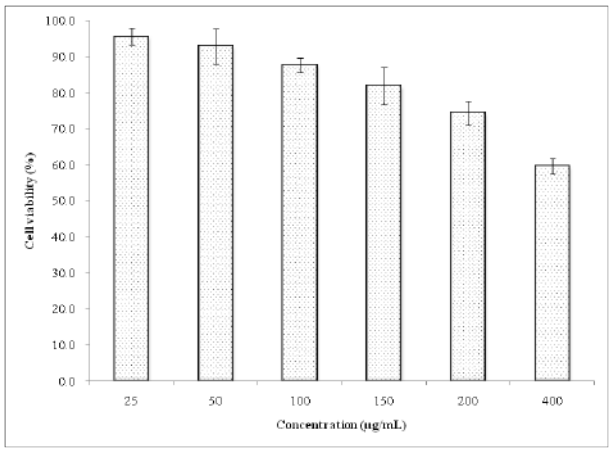

Fig. 3. Cell viability of methanol extract of *D. atrovirens* bark against RAW 264.7 cells.

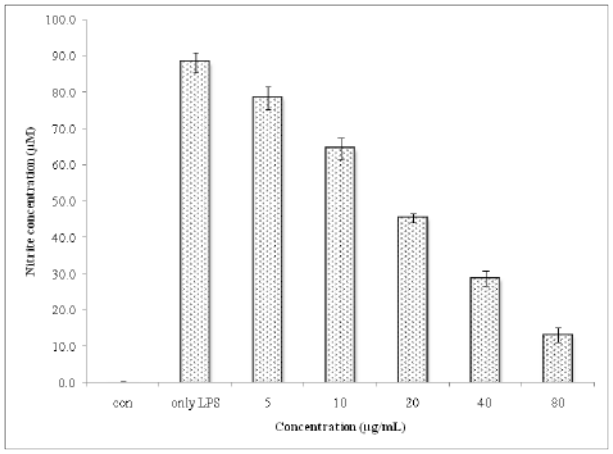

Figure 4. Effect of methanol extract of *D. atrovirens* bark on nitric oxide production in LPS-induced RAW 264.7 cells.

RAW 264.7, a murine macrophage cell line has been frequently used for the screening of anti-inflammatory drugs. The results of the present study demonstrated that the methanol extract significantly decreased the nitrite accumulation in LPS-stimulated RAW 264.7 cells in a concentration dependent manner. NO is a multifunctional signaling molecule, thus the impact of the extract or compound on NO production likely has further effects on signaling pathways in many cell types (30). Epidemiological studies have shown a positive correlation between consumption of plant foods, which are rich sources of antioxidants, and reduction in risk of diseases mediated by reactive oxygen species. Previous studies have also suggested that plant secondary metabolites act as excellent anti-inflammatory agents and they play an important role in oxidative stress and inflammation (31-33).

4. CONCLUSION

The extractable total phenolics, and flavonoids were found to be higher in the methanol extract of *D. atrovirens* bark and the methanol extract of*D. atrovirens* bark manifested the strongest radical scavenging activities. The methanol extract of *D. atrovirens*bark also significantly decreased nitrite accumulation in LPS-stimulated RAW 264.7 cells indicating that they potentially inhibited the NO production in a concentration dependent manner. Further studies are warranted in relation to the mechanism of action of bioactive components from the methanol extract of *D. atrovirens* bark.

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