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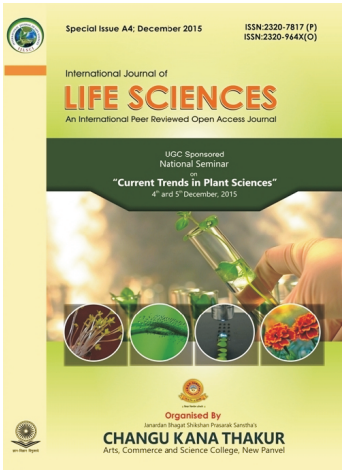

UGC Sponsored
National Seminar on

Current Trends in Plant Sciences

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Message

Janardan Bhagat Shikshan Prasarak Sanstha's Changu Kana Thakur College of Arts, Commerce and Science stands for quality education. We believe that education plays a vital role in building a healthy and progressive society by moulding students into rational, tolerant and empowered citizens. We at Changu Kana Thakur College strive to provide students with quality education that will enable them to aim for the best through an amalgamation of attitude and acumen.

I congratulate the department of Botany for taking a step towards furthering our mission by organizing a National Level Seminar on 'Current Trends in Plant Sciences'. Plant Sciences, I believe holds the key to solving many if not all the problems that trouble mankind today. Advances in Plant Sciences will play a major role in revolutionizing technologies in medicine, agriculture and sustainable development in the near future.

I am confident that the Seminar will serve to bring together on a common platform, young researchers, teachers, eminent Scientists and industry personnel. A healthy exchange of ideas and views and the intellectual deliberations will benefit all the participants, further contributing to the subject.

I wish all success to the Seminar.

Date: 20th November 2015

Place: New Panvel

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Message

Dr. S.T. Gadage
Principal

I am happy that the department of Botany is organizing a Two Days National Seminar on 'Current Trends in Plant Sciences'. This seminar is one more confident step of our College in achieving our ambitious goals and fulfilling our mission. Our College has always been sensitive towards the significant changes that are taking place in the field of higher education. We have responded positively to these changes by ensuring that our learners benefit the most from them. We have in the past, and we always will remain committed towards providing our learners the best education, that will result in their growing into confident, successful, skilled and cultured citizens of our society.

The main aim of this Seminar is to bring about a constructive dialogue between the teaching fraternity, researchers, students of Botany, industry personnel and scientists. I am sure that these deliberations will prove to be beneficial to the learners and the young researchers as well as the teachers.

I am sure that the discussions in the seminar will inspire the learners, teachers and research scholars participating in the seminar, to develop themselves as researchers. Exposure to various arenas of Botany would definitely help them to enhance their knowledge and skills in their chosen area of expertise. Successful completion of this seminar will help to achieve academic up-gradation of the educational institute.

I wish all the best for successful completion of this seminar.

C.K.T.A.C.S. COLLEGE NEW PANVEL

Date: 20th November 2015

Place: New Panvel

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UGC Sponsored National Seminar on

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INVITED TALK

KEY NOTE ADDRESS

The Medicinal the Indigenous Bioresource for our Health Care

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It is generally recognized that the habitats with higher species richness are expected to have high proportion of medicinal plants. In addition, within the habitat and ecosystem, presence of more microsites results in the occurrence of high number of plants with secondary metabolites. In agreeing with this concept, India being a country with different agro-climatic and bioclimatic regions harbors rich diversity of plants including many medicinal plants. Due to this fact, India attains the status of one among the 17 mega-diversity countries in the world. The floristic documentation studies revealed that in India, approximately 17500 angiosperm species are present due to isolated climate and other environmental factors, about 5000 endemic plants are distributed in various parts of our nation. The threatened plants are reported to be around 800 all over India.

The availability of high plant diversity since long back, turned the people of India to follow the traditional system of medicinal practices like ayurveda, sidda, unani and Tibetan which are mainly constituting the formulation of plant products. A huge number of nearly 400000 practitioners of all these traditional systems are available in India with 6780 pharmacies and it is known that 80% of drugs for these practices/ pharmacies are the plant products. In ayurveda system, only 370 plants are effectively used in our country. In addition, 10 species as under mentioned alone are listed as top traded medicinal plants:

S. No.	Botanical Name	Common Name	Avg. 3 yrs (99-00, 01-02, 04-05) (tons)
1.	*Emblica officinalis* Gaertn.	Amla	26,553.03
2.	*Asparagus racemosus* Willd.	Shatawar/ Satawar	11, 943.17
3.	*Saraca asoca* Roxb.	Ashoka	7,702.43
4.	*Cassia angustifolia* Vahl.	Sonapatri/Sana	7,498.67
5.	*Withania somnifera* Dunal.	Aswagandha/Asgandh	7,353.77
6.	*Azadirachta indica* A.Juss.	Neem	7,129.67
7.	*Terminalia chebula* Retz.	Harar/Halela Zard	6,038.23
8.	*Aegle marmelos* Corr.	[1] Bael [Bark] [2] Belgiri	5,648.50
9.	*Adhatoda vasica* Nees.	Adusa/Arusa	5,485.80
10.	*Sida cordifolia* Linn.	Kanghi	5,407.77
	Average GR	15.1% (2001-02)	16.7% (2004-05)

In recent year, owing to awareness on medicinal plants, at global level the tremendous increase in the sale of herbal products has been recorded which indicates the growing popularity of herbal therapies. This trend provides new opportunities for the countries like India to establish many herbal industries.

The increased demand of medicinal plants leads to over exploitation of wilds that threatens the survival of not only the rare species but also enormous number of commonly available plants. Further, the developmental activities, ecosystem destruction, excessive urbanization, vast deforestation and population explosion are the other factors for the rapid depletion of medicinal bio-resources. Hence, people and biotic conservation authorities must concentrate in this direction for sustainable utilization and conservation of biodiversity in general and medicinal plants in particular.

As mega-diversity country, India has tremendous scope in the area of medicinal plants. However, the further prospect of our medicinal plant resource base is depending upon our knowledge and availability of information about the following aspects:

- Distribution and availability
- Traditional uses and their validation
- Pharmacoepeial standards
- Chemical and genetic diversity
- Basic reproductive biology and microbial associations
- Domestication, nurseries and raising plants.
- Conservation and cultivation practices (Good Agricultural Practices, GAP)
- Post – harvest technology and value additions (Good Manufacturing Practices, GMP)
- Understanding metabolic pathways for secondary product synthesis
- Status of bioprospection of the biomolecules and genes from the medicinal plants
- Deploying genes and metabolic pathways in heterologous systems

Net working advantages

- Easy and quick access and sharing data and information including access to genetic resources and associated Traditional Knowledge (TK) and technologies within and outside the country.
- Conservation, management and sustainable use of plant resources including bioprospe cting, value addition to genetic resources and associated TK.
- Management of protected areas including heritage sites
- Implementation of fair and equitable benefit sharing
- Informed decisions on various aspects of medicinal plants.
- Capacity building and human resource development in participating institutions in medicinal plant diversity conservation, documentation, bioinformatics, bioprospec-ting, policies etc.
- Trade and marketing of medicinal plants and plant products.
- Protection of sovereign rights and IPRS on plant genetic resources and associated TK.
- Development of national legislations and policies on medicinal plant diversity.
- Implementation and monitoring of various international legal policies and strategies pertaining diversity within the territorial jurisdiction of the country.
- Linkage of the national network with international databases and networks of biodiversity and related policy and legal frameworks (e.g. CBD, WTO, FAO, PCT, etc.).

Domestication and Development of agro-techniques

For the conservation of wild plants of medicinal importance and their genetic stock, the World Trade Organization favors the farm product materials rather than wild souces. Domestication

of wild species involves the steps *viz.,* research and development aspects of domestication, standardization of agro-technology and post harvest technology with the details as below.

Still further exploration of our plant resources in terms of taxonomy, ecology, phytochemistry *etc* is essential to have comprehensive understanding about our biodiversity on value and conservation basis.

Domestication	**Cultivation Package**	**Package Pot-Harvest Technology**
Phenology	Cutivar	Drying
Seed biology	Date of sowing/planting	Processing
Micropropagation	Method of planting	Formulation
Nursery management	Spacing	Packaging
	Intercultural	Labeling
	Crop protection	Transport
	Harvest time and method	Storage
	Integration in agro-systems	

***

KEY NOTE ADDRESS

Phytoremediation in Eco-Restoration

Chaphekar Sharad B

Retired Professor, Environmental Botany, University of Pune, Pune, India

Aim of any eco-restoration activity is to convert a derelict habitat into an environmentally acceptable, productive and sustainable ecosystem. The restored ecosystem may or may not be 'natural'. Since eco-restoration is an expensive process in terms of time, manpower and finances, it is necessary that the end use of restored habitat is decided at the beginning of the eco-restoration effort.

With involvement of students, some efforts were made to improve degraded habitats, using plants; though it must be emphasized that restoration is a team work involving expertise from several disciplines in sciences and humanities. Some examples of related research and action work undertaken are presented here. They are: Development of Green Belts in areas suffering from air pollution, Conversion of a stone quarry into a recreation water body, Stabilization of toxic tailings from metal mining process, Treatment of fly ash from thermal power plant, etc.

Attempt in all these projects, including some not presented here due to limitations of time, is to improve greenery and add to carbon sequestration potential of the affected areas.

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KEY NOTE ADDRESS

Ayurved and Aahaar (Nutrition)

Bagool RG

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AYURVEDA means the way of living. It tells how to live, obviously a healthy life. If something is wrong with the health, then how to restore it by altering the routine, change in AAHAR or by administering some medicines. Some of the basic principles of AYURVED are:

PANCHMAHAABHOOTA concept and TRIDOSH concept.

AYURVED considers nutrition i.e. AAHAR as the most important factor for healthy life followed by SVAPNA (NIDRA or sleep) and BRAHMACHARYA (observance of celibacy i.e. complete sexual abstinence). Together, they are called UPSTAMBHAS or TRIAD.

A proper, skillful and optimum use of this triad leads the human body to maintain its integrity being rich in BALA (physical and immunological strength), VARNA (complexion) and UPCAYA (growth and nourishment) throughout the life.

The triad assists the three STAMBHAS (the pillars) or DOSHA viz. KAPHA, PITTA and VATA in the bodily activities. They originate from the PENTAD or the PANCHMAHABHOOTAS namely, PRUTHVI (the Earth), AAPA (water), TEJ or AGNI (energy or fire), VAYU (air) and AAKASH (space). VATA dosha is produced by space and air; PITTA dosha is composed of water and energy; KAPHA dosha is formed by earth and water.

The kapha molecules are heavy, stable, soft, slimy and moist; pitta molecules are hot, light, clear, slightly viscous and have a penetrating power; vata molecules are light, minute, clear, rough and dry.

The three doshas perform specific functions in the body.

There is a cyclic change of dominance of the kaph-pitta-vata trio during the life span of a person and also during change of every season and every day. The cycle represents synthesis (kaph), maturity (pitta) and degradation (vata).

The next important concept is the AGNI and AAMA.

AGNI means fire. It refers, specifically, to the digestive powern i.e. the JATHARAAGNI the digestive enzymes and the metabolic fire. If the digestion is incomplete or abnormal, it leads to formation of toxic substances or the AAMA. When there is mandagni (weakening of digestive power or AGNIMANDYA), the aama gets absorbed in the RASADHATU; it can cause variety of diseases from acute diarrhea, dysentery to chronic problems like rheumatoid arthritis, ulcerative colitis, diabetes, cirrhosis of liver etc. Hence, while treating any disease arising out of increased AAMA, the treatment is first directed to eliminate the cause by increasing AGNI to its normal level.

AAHAR or NUTRITION

पंचभूतात्मके देहे आहारः पांचभौतिकः।
आहार संभवं वस्तु रोगश्चाहार संभवाः॥

We are what we eat. Somebody has said " *Anna Taari , Anna Maari, Anna Nana Vikaari"*, means food provides nourishment and saves a person; food is the cause of death and food is the cause of various diseases.

The modern science says that the food to be consumed must be balanced. It must contain all the important ingredients viz. carbohydrates, fats, proteins, salts and vitamins in proper proportion. The digestion of proteins, fats and carbohydrates give amino acids, fatty acids & glycerol and sugars, respectively. Ayurvedik scientists consider them as the end products of the kaph, pitta and vata, respectively. These constitute the major part of the RASADHATU. According to Ayurveda the food must contain all the six tastes viz. MADHUR(sweet), LAVAN(salt), AMLA (sour), KATU (pungent or hot), TIKTA (bitter) and KASHAYA (astingant).

In order to maintain good health, proper and healthy food should be consumed in proper quantity and at proper time. Charaka has suggested eight main criteria for deciding the intake of food under ASHTA AAHAAR VIDHI VISHESHAAYATAN. These are PRAKRUTI (natural qualities of the food), KARAN (method of preparation or SANSKAR), SANYOG (combination of two or more substances), RASHI (quantity of food or MATRA), DESH (the area where the food substance is produced and also where the food is being consumed), KALA (the time of consuming food), UPYOG SANSTHA (rules of consuming food) and UPYOKTA (the consumer, his prakruti, his physical and mental status etc.).

Change of life style, general ignorance about the hygiene and faulty food habits, excessive consumption of fast/junk food have made the life miserable .The science has given a new concept to the world, namely NUTRACEUTICALS. The term was coined in the twentieth century. However, it has its roots in Ayurveda and contemporary sciences which are, still being practiced in India, China and Arabian countries since more than thousand years.

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KEY NOTE ADDRESS

Role of plant tissue culture in cloning, improvement and germplasm conservation of some commercial *Citrus* spp.

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Amongst fruit trees, Citrus may be considered as the number one fruit of the world and number three fruit of India after mango and banana in view of its nutritional and medicinal value as well as its production. Despite North-Eastern India being the native place of citrus, a huge orchard area, rich germplasm and favorable agroclimate, the Citrus fruit production in India has been declining at an alarming rate during the past several decades mainly due to destruction of orchards by diseases (particularly viral) and general neglect towards replenishing the declining orchards with certified healthy and high productive varieties of *Citrus*. The poor state of Citrus Industry obtained in the country can greatly be alleviated or even be changed to a situation, in which India becomes a major citrus producing country in the world with the application of Biotechnology, precisely Plant Tissue Culture.

Citrus tissue culture has been initiated for the first time in India at Delhi University, Delhi during late 1950s by Late Prof. P. Maheshwari, the Father of Plant Tissue Culture in India. However, at National Botanical Research Institute, Lucknow, the journey of Citrus Tissue Culture began during late 1960s and the first Citrus tree from *in vitro* culture has been produced in 1972. Afterwards, the journey continued and remarkable success achieved in various important aspects of Citrus Tissue Culture research, like, shoot meristem culture, unpollinated pistil culture and micrografting for virus elimination; haploid production through androgenesis for genetic improvement; production of cloned plants of important scion species, namely, *C. aurantifolia* (lime), *C. sinensis* (sweet orange), *C. reticulata* (orange or mandarin) and *C. nobilis* x *C. deliciosa* (Kinnow Mandarin) through nodal stem segments; production of cloned as well as disease-free rootstocks, namely, *C. karna* (Karna Khatta), *C. jambhiri* (rough lemon), *C. limonia* (Rangpur lime) and *Poncirus trifoliata* by exploiting nucellar polyembryony. Under multilocational field trials, the *in vitro*-raised *C. aurantifolia* plants exhibited better performance. Besides, efficient *in vitro* processes for germplasm preservation of aforesaid *Citrus* spp., including *C. indica* (an endemic threatened wild relative of citrus) were developed for establishing 'Germplasm Repositories' of *Citrus* spp. growing in diverse agroclimates.

***

INVITED TALK

Plant Resources of Arid zones-National assets in the Anthropocene: Applications of technologies for sustainable utilization (the Sustainocene)

Shekhawat NS, Kataria Vinod, Shekhawat Smita, Patel AK, Rai MK, Phulwaria M, Kaur G, Singh RP, Rathore JS, Panwar Deepak, Agarwal Tanvi, Dagla HR, Sharma Udit, Choudhary D and Shekhawat Kanwar J

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In Praise of Plants (Elope de la plante, Timber Press, Portland/London, 2002) Francis Halle (English edition) on page 293 (Who needs the other most?),writes "Remove the fauna from a forest, leaving only the plants. What will happen? Nothing - for years, perhaps centuries. Plant will continue to grow normally." On page 294, the author writes, "Now remove all the plants from a forest, leaving only the fauna. There is no doubt about the outcome. Wait several days, even several hours, for the nightmare." "All animals disappear once deprived of plants. Bacteria and fungi studiously will take care to clean up all that can be in good time". All We Are Saying Is Give Plants a Chance! States Peter M. Gresshoff { Journal of Biomedicine and Biotechnology , 2:3 (2002) 115–116} because Every year green plants fix 56,000,000,000 tons of Carbon-dioxide and produce 170-200,000,000,000 tones of biomass for us.

There is no Nobel Prize for plant science, preventing the popular press from featuring achievements in plant biology/Botany (since the inception of Nobel Prizes one century ago, only three awarded for plant-related research). How sad it is? We all accept that plants are important in economical, environmental, and ecological terms. Imagine a world without flowers, shade trees, wooden furniture or floors, riverbanks without trees, mountains without meadows, bakeries without bread, glasses without wine/fruit juice(s), and grocery shops without fruits and vegetables and air without oxygen. Plants also tell us about biology. Most plant cells are pluripotent, indeed totipotent, and the growth of the soma occurs from clusters of stem cells called meristems, from which all organs and cell types derive. Mendel discovered the basic laws of genetics not with bees but peas. The first cloning from a differentiated somatic cell involved not Dolly but tobacco [Dolly and all others like her are not really clones as the mitochondrial DNA is still derived from the egg donor; they are nuclear transplants] by Vimla Vasil and A.C. Hildebrandt. The first recognized gaseous hormone was not nitrous oxide but ethylene. The first discovered morphogen was not retinoic acid but cytokinin. Catalytic RNA (now ribozyme) was first suggested in viroids attacking avocado. Transposable elements were discovered in corn before bacteria, yeast, and fruit fly. Plant development offers an alternative paradigm for development as most organs are induced post

embryonically, relying on the precise perception of chemical signals (Gresshoff, 2002). The ndosperms tissues that feed us are examples of programmed cell death and Gene/genome imprinting. Genome silencing/cross protection and genetic engineering in nature were discovered in plants. Now, we know TALEs (transcription activator like effectors), these are products of plant pathogens and are effectively used for genome editing/gene drive/genome surgery along with ZFN and Mega-nucleases and CRISPR-Case systems. Genome editing will probably bring changes in the fields of biotechnology/medicine and other fields beyond our imagination. There is already heated debate on its applications and regulations.

Climate change and global warming are caused by human activities (anthropogenic) and hence modern era of climate change is described as "Anthropocene"-the slice of Earth's history during which human have become a major geological force (Monastersky, R., Nature, 519, 12 March,2015,pages 144-147).Through mining activities alone, humans move more sediment than all the world's rivers combined. We, the humans warmed the planet, raised sea levels, eroded the ozone layer and acidified the oceans. People around the world are trying to figure out the options humanity has for changing course. There are many answers but need is for emphasis on solutions. Achieving goal of limiting the rise in global average temperature is main objective. This requires political leadership and will(s) which will be neutralized by very active opposition by entrenched industrial/commercial interests. It is not easy to transform global or local economy and industrial bases for growing and ever-demanding (much of which is suffering from poverty; still want all the modern conveniences and comforts) population.The world needs solutions and full set of technologies that are not only cost-effective but also socially and politically viable (Nature editorial,15th October, 2015).

The Holocene ("recent whole") period began 10,000 years ago and from that time till about 1800 CE. It was term given to the post-glacial geological epoch by the International Geological Congress in Bologna in 1885. Land ecosystems (mostly wild) were converted to mostly anthropogenic by the mid 20th century. It has been argued that human activities have pushed the planet earth from Holocene into Anthropocene period that has five dominant characteristic features namely, population, poverty, preparation for war, profit and pollution. The present destructive impact of what may be termed the corporate-military complex on the biosphere is great scientific challenge and must be focus of research and policy.

The term "Sustainocene" was coined by Australian physician Bryan Furnass in 2012. It refers to a period where governance structures and scientific endeavors coordinate to achieve the social virtues of ecological sustainability and environmental integrity. Thomas Faunce (2015) has suggested an idea of making all the profusion of human structures on the Earth's surface do photosynthesis {in most cases without biology-the artificial photosynthesis (AP)}.The vision of globalized AP technology supporting the humanity acting as ecosystem steward in a Sustainocene epoch and fostering traditional and emerging individual and social virtues. As on day green plants are only hope for maintaining ecosystems/ecosystem services and for keeping the climate and earth hospitable for living organisms. We must enjoy working on plants and must learn how to survive when Mother Nature is hostile? Adansonia digitata and *Zizyphus jujuba* s urvive for 1,000 years; redwood for 2, 000, bristlecone pine for 5, 000, clones of aspen for 10,000 and clones of huckleberry for 13,000 years. The Great Banyan is a banyan tree (Ficus benghalensis) located in Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah, near Kolkata. It is the widest tree in the world in terms of the area of the canopy and is estimated to be about 1200 to 1250 years old

The Indian subcontinent lies at the same latitude as the great desert region to its west, yet India is not a desert. The yearly monsoon-seasonal winds bring moisture from the ocean to land and carry life-supporting rains. The Harappans, the most advanced ancient civilization in the Indus River basin flourished here. This oldest known urban civilization thrived on the northwest edge of the Thar Desert. Then around 3,800 years ago the Harappan civilization began to crumple, after few centuries it was gone. It is suggested that subtle ancient environmental condition and a weakening of monsoon doomed the Harappa. This also shows that people can be vulnerable to a slowly changing climate, whether natural or human-induced (anthropogenic). The Thar Desert is especially dry due to hot winds from the Middle East that often block the monsoon (battle ground for monsoon and westerly hot and dry winds). This desert is vast tract covering over 4000 sq.km. It stretches from the western fringes of Aravalli Mountain Range (AMR). Much of it has a rainfall of 100-300 mm, though eastern end of arid zone lies to the 400 mm mean annual rainfall. The Aravalli Mountain Range literally meaning "line of peaks" is approximately 692 km in northeastern direction across Gujarat, Rajasthan, Haryana, and Delhi. It is one of the world's oldest mountain range. It has been suggested that the Indian Desert is a meeting place of Eastern and Western flora. It has Tropical, African, Iranian, Sindo-Rajasthani (endemic), Oriental and Australasian elements. The flora has overall dominance of African elements (37.1%) (Bhandari,1990). Endemic flora is 9.4 percent. The variability in the climatic, edaphic and topographic conditions causes diversity in the vegetation. There are hardy trees, shrubs and grasses. The state of Rajasthan is rich in floral diversity as around 1911 wild species belonging to 780 genera and 154 families grow over here (Shetty and Singh, 1987-93). *Ephedra foliata* is the only gymnosperm. Besides, these about 275 species are grown by the natives for grains, fruits, vegetables, fodder and other purposes. Some of these plants bear unique botanical characters and harbor plenty of microbes that are becoming important in era of genomics/metagenomics and proteomics for prospecting and further explorations. Many of these plant yield herbal medicines, nutraceuticals and products of special interest for industry and society. There is need for characterization and conservation/propagation of germplasm(s) of these plants. Methods of molecular biology, biotechnology, nanotechnology/ bionanotechnology and gene/genome editing can be utilized. During the last 40 years our group at the Department of Botany, Jai Narain Vyas University, Jodhpur with the support from DBT, DST, DoEn & forest and Climate Change, CSIR, UGC and other agencies developed and applied biotechnological approaches. These will be discussed during the presentation of this paper.

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RESEARCH ARTICLES

RESEARCH ARTICLE

Pharmacognostical, Physico-chemical and Phytochemical Evaluation of leaves of *Cassia tora* and *Cassia fistula*"

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Abstract

Cassia sp. is well known in Indian system of medicine for their huge medicinal properties. In the present study, pharmacognostical, physico-chemical and phytochemical characters of leaves of Cassia tora and Cassia fistula were studied. Microscopic and fluorescence characters of powder of the leaves were studied for identification and characterization of various features. Physicochemical parameters like Foreign Organic matter (*C. tora* 1.5% ± 0.005; C. fistula 1.0% ± 0.002), Total Moisture (*C. tora* - 0.7% ± 0.002; C. fistula -1.5% ± 0.07), Ash Values (total ash, acid insoluble ash, water soluble ash, sulfated ash) and Extractive values of leaves of both the plants were evaluated. Heavy Metals viz. As, Cd, Cr, Pb, Hg were found in permissible limit i.e. less than 0.01ppm in both the leaves of Cassia species. Zinc, an essential micronutrient, was also found in permissible limit (*C. tora* -23.67ppm; C. fistula- 5.07ppm). Phytochemical screening showed the presence of phenols, flavoniods, tannins, carbohydrates and triterpenoids in the polar solvent extracts of both the plants. TLC finger print profile was also studied by observing the developed and derivatized TLC plate under UV (254 and 366nm) light. The present study reveals specific characteristics of the particular plant materials that can have a significant use in identification of crude samples and quality assessment of raw product i.e. impurities and adulterations, which can serve as a reference for further investigations.

Keywords: *C. tora*, *C. fistula*, Pharmacognostical evaluation, Phytochemical screening, Physicochemistry.

INTRODUCTION

Cassia Linn. (Family – Caesalpiniaceae) is a large tropical genus with about 600 species of herbs, shrubs and trees; some of which are

widely distributed throughout the world especially in tropical countries and is abundantly available in India. Most of the plants of genus are well known in Indian system of medicine for their cathartic, purgative, antiparasitic, anti-helminthic, antifungal, antimicrobial, anti-inflammatory property etc.. Various plants of *Cassia sps.* are also used traditionally in the treatment of periodic fever and malaria in subtropical and tropical regions. Some plants of this genus are widely used as traditional medicine in Africa and India for the treatment of ulcers. Several of them yield timber, dyes, fodder, vegetables, edible fruits etc.. In some places seeds are used as substitute for coffee (Dave and Ledwani, 2012). Plants of *Cassia* genus are rich source of polyphenols, flavonoids, tannins, mucilage, polysaccharide, steroids, anthraquinone glycosides and derivatives of anthracene (Sanghi *et al.*, 2006). The anti-inflammatory activity of *Cassia* may be attributed to the flavonoid molecules present in them (Ganapaty *et al.*, 2002).

Cassia fistula Linn. (Indian Labernum), a semi-wild tree (20–30 ft.) also known as the Golden Shower, has become extensively distributed in various countries including Mauritius, India, South Africa, Mexico, China, etc. and is used as an ornamental tree for its beautiful bunches of yellow flowers (Mukhopadhyay *et al.,* 1998). Leaves contain free rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) and its glycosides – Sennosides A & B (Thirumal *et al.*, 2012). Leaves and flowers are both used as a purgative drug. Juice of leaves is useful as dressing for ringworm, relieving irritation and relief of dropsical swelling (Danish *et al.,* 2011).

Cassia tora is an annual herb also known as Wild Senna. In India, it occurs as waste land rainy season weed. *C. tora* leaves contain emodin, stigmasterol, β-sitosteral-β-D-glucoside, freindlen, palmitic, stearic acid, succinic, d-tartaric acids and derivatives of quercitrin. According to Ayurveda the leaves and seeds are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardiotonic, expectorant etc. and they are useful in leprosy, ringworm, colic, dyspepsia, constipation, cough, bronchitis and cardiac disorders (Kawade and Vite, 2013).

The main objective of the present investigation is to study and compare pharmacognostical features and physiochemical constants along with phytochemical screening of the leaves of these two species as very less information is available on these parameters.

MATERIALS AND METHODS

The fresh leaves of *Cassia tora* (*C. tora*) and *Cassia fistula* (*C. fistula*) were collected from the suburban region of Maharashtra and they were identified and authenticated from the Blatter Herbarium, St. Xavier's College, Mumbai (Maharashtra). The leaves of both plants were separated from twigs and shade dried. Later they were crushed into coarse powder (sieve no. 10/44) and kept in properly labeled air tight containers. All chemicals used in assays were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck Co. (Santa Ana, CA, USA). All parameters studied under pharmacognostic features were followed from guidelines' of American Herbal Pharmacopoeia, AHP – Botanical Pharmacognosy, 2011; Khandelwal, 2008; Indian Pharmacopoeia, 2007; WHO, 1998 and British Pharmacopoeia, 1980.

Macroscopic evaluation:

The macroscopic evaluation of leaves of *C. tora* and *C. fistula* was done by observing the external characters like shape, size, texture, surface characteristics and fractured surface with the help of magnifying lens. The organoleptic features like colour, odour, taste, feel and fracture of the crude drug were observed with sensory organs and compared.

Microscopic evaluation:

Microscopical studies were carried out from transverse sections (T.S.) of fresh leaflets. The sections were doubled stained with saffranine and fast green. Mounted in 50% glycerine and

observed under Lawrence & Mayo monocular (LM–52-1602) and Phase contrast-trinocular microscope (LM–52-1802). Microphotographs of sections were taken for the identification of various tissues and their arrangement. Characteristic features of leaves of *C .tora* and *C. fistula* were noted for comparison.

Determination of Physicochemical Parameters:

Following physicochemical parameters were determined in coarsely powdered leaves of *Cassia species* as per standard procedures (Khandelwal, 2008; Evans, 2002 and WHO, 1998).

Determination of Foreign Organic Matter:

100–500 g of the crude samples were taken, weighed and spreaded in a thin layer. The foreign organic matter (FOM) was detected by the use of a lens (6x). Separated FOM weighed and percentage of presence was calculated.

Total Moisture content:

1g air-dried coarse powder of leaves of *C. tora* and *C. fistula* were weighed in previously tarred crucible and dried at 105°C in hot air oven and cooled. Total moisture was calculated with respect to difference on pre-dried and post dried weight of sample.

Ash Value:

Total ash, acid insoluble ash, water soluble ash and sulfated ash were determined in the powdered leaves of *C. tora* and *C. fistula* according to the standard procedure.These values are used for determining the quality and purity of the powdered form of crude drug.

Extractive values:

About 5 g of dried powdered leaves of *C. tora* and *C. fistula* were weighed and macerated for 24 hours with 100 ml of solvents (ethanol 95% and water) in a glass stopper flasks. The flasks were shaked frequently for six hours and allowed to stand for next 18 hours. Extracts were filtered; 25 ml of extract was transferred in tarred dish and evaporated to dryness on water bath. The dried extract was further kept in hot air oven at 105°C, cooled and weighed. The percentage of extractive values for different solvents was calculated.

Heavy Metal analysis:

The objective was to determine the essential and non-essential heavy metals and their amount in the leaves of plants *viz. C. tora* and *C. fistula*. Selected heavy metals *viz.* Mercury (Hg); Arsenic (As); Lead (Pb); Zinc (Zn); Chromium (Cr) and Cadmium (Cd) were analyzed from SAIF (Sophisticated Analytical Instrument Facility) Department of IIT, Powai (Maharashtra, India). The samples were digested by wet digestion method and analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) and amount of heavy metals were quantified in the leaves.

Foaming index:

1g powder of leaves of each plant was weighed and transferred to a conical flask containing 100 ml of boiling distilled water. Moderate boiling was maintained for 30 min., cooled and filtered into volumetric flask and the volume was made up to 100 ml with distilled water. The decoction was transferred into stopper test tubes in successive portions of 1 ml, 2 ml, 3 ml and up to 10 ml and volume was made up to 10 ml in each tube with distilled water. Tubes were shaken in length wise motion for 15 seconds and were allowed to stand. The height of foam in each tube was measured.

Swelling index:

1g of coarse powder of leaves of *C. tora* and *C. fistula* was taken in glass- stopper measuring cylinder. 25ml of water was added and shaked occasionally for 1 hour and kept for 3 hours. Swelling index was calculated by measuring the volume in ml occupied by the 1 g swollen drug.

pH Determination :

pH of extracts (5%) of leaves of *C. tora* and *C. fistula* was determined by a standard calibrated pH meter and values were recorded.

Fluorescence analysis of powdered drug :
Fluorescence powder drug analysis of the crude powder of *C. tora* and *C. fistula* was carried out in the UV (Ultra-Violet) light as per the method of Chase and Pratt (1949). The fluorescence patterns were obtained when the powdered drug reacted with different chemical reagents. The identification and comparison of the colors was done using the standard colour index chart.

Preliminary phytochemical screening:
Extracts of *C. tora* and *C. fistula* leaves were prepared in different solvents (Ethyl acetate, Chloroform, Acetone, Petroleum Ether, Methanol, Aqueous) by using condenser and subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids.

TLC profile of hydro-alcoholic extract:
The TLC profile of hydro-alcoholic extract was carried out by using Ethyl Acetate : Formic Acid : Glacial Acetic Acid : Water (100 : 1.0 : 1.0 : 28 v/v/v) solvent system (Wagner *et al.*; 2009).The visualization of spots was done by observing the plate under UV light (both long and short) and after derivetizing with 10% ethanolic NaOH spray reagent. Color for different spots was observed and recorded.

RESULTS AND DISCUSSION

Evaluation of different parameters *viz.* pharmacognostical features, physicochemical constants and phytochemical screening of the leaves of *C. tora* and *C. fistula* Linn. was carried out and discussed below:

Macroscopic evaluation:
Morphology is the pre-requisite variable for identification of any matter. Here, the leaves of *C. tora* and *C. fistula* Linn. were differentiated on the basis of size, shape, apex, base and presence or absence of gland and flexible spine (Table -1 and Fig.– 1.A).

The leaves of *C. fistula* have symmetric base without gland; whereas leaves of *C. tora* have asymmetric base with gland. In the *C. tora* main rachis has conical gland between the last two pairs of leaflets. Another differentiating character was presence of flexible spine on the dorsal surface near lowermost pair of leaflets in *C. tora* however it is absent in *C. fistula* (Cooke, 1967).

Microscopic Evaluation:
Microscopic evaluation of the plant is essential to identify the adulterants and for the correct identification of the plant. The results of microscopic evaluation are given below:-

Transverse section of leaflet:
T.S of leaflet through midrib and lamina showed dorsi-ventral structure and was covered by epidermis on both the surfaces. Upper and lower epidermis was further covered by cuticle. Below the epidermis, single layer of elongated palisade cells was observed followed by 3-4 layers of loosely arranged spongy parenchymatous cells in both the species of *Cassia*.

In *C. tora,* epidermis showed uni-to multicellular uniseriate trichomes with constricted uppermost cell. The midrib comprises of parenchymatous tissues embedding vascular tissues. Vascular tissues were randomly scattered in centre and surrounded by sclerenchymatous cells [**Fig. I (a–e**)]. Calcium oxalate crystals were observed in the centre of midrib within parenchymatous tissue (**Fig. I (f**).

In *Cassia fistula,* adaxial epidermal cells are mostly rectangular to square in shape and in some cases; it was polygonal [**Fig. II (g-l**)]. Non-glandular, unicellular trichomes were found on surface of epidermis. In the centre of midrib, vascular bundles were arranged in specific ring manner with radial clusters of xylem elements.
In both the species of *Cassia,* Paracytic stomata were present on both the surfaces of leaves with maximum stomata on abaxial or lower surface.

Table 1: Macroscopic description of Leaves.

Sr. No.	Parameters	Description	
		Cassia tora	***Cassia fistula***
1.	Arrangement	Paripinnate Compound	Paripinnate Compound
2.	Leaflets	3 pairs	4 – 8 pairs
3.	Apex	Obtuse to Slightly reduce	Acute
4.	Shape	Obovate – Oblong	Ovate – Oblong
5.	Margin	Entire	Entire
6.	Length	3 – 4 inch	9 –16 inch
7.	Colour	Green	Green
8.	Stipules	Long	Minute
9.	Base	Oblique /Asymmetrical	Symmetric
10.	Texture	Smooth	Coriaceous & Leathery
11.	Mid-rib	Biconvex and less prominent on either side	Biconvex and more prominent on lower side

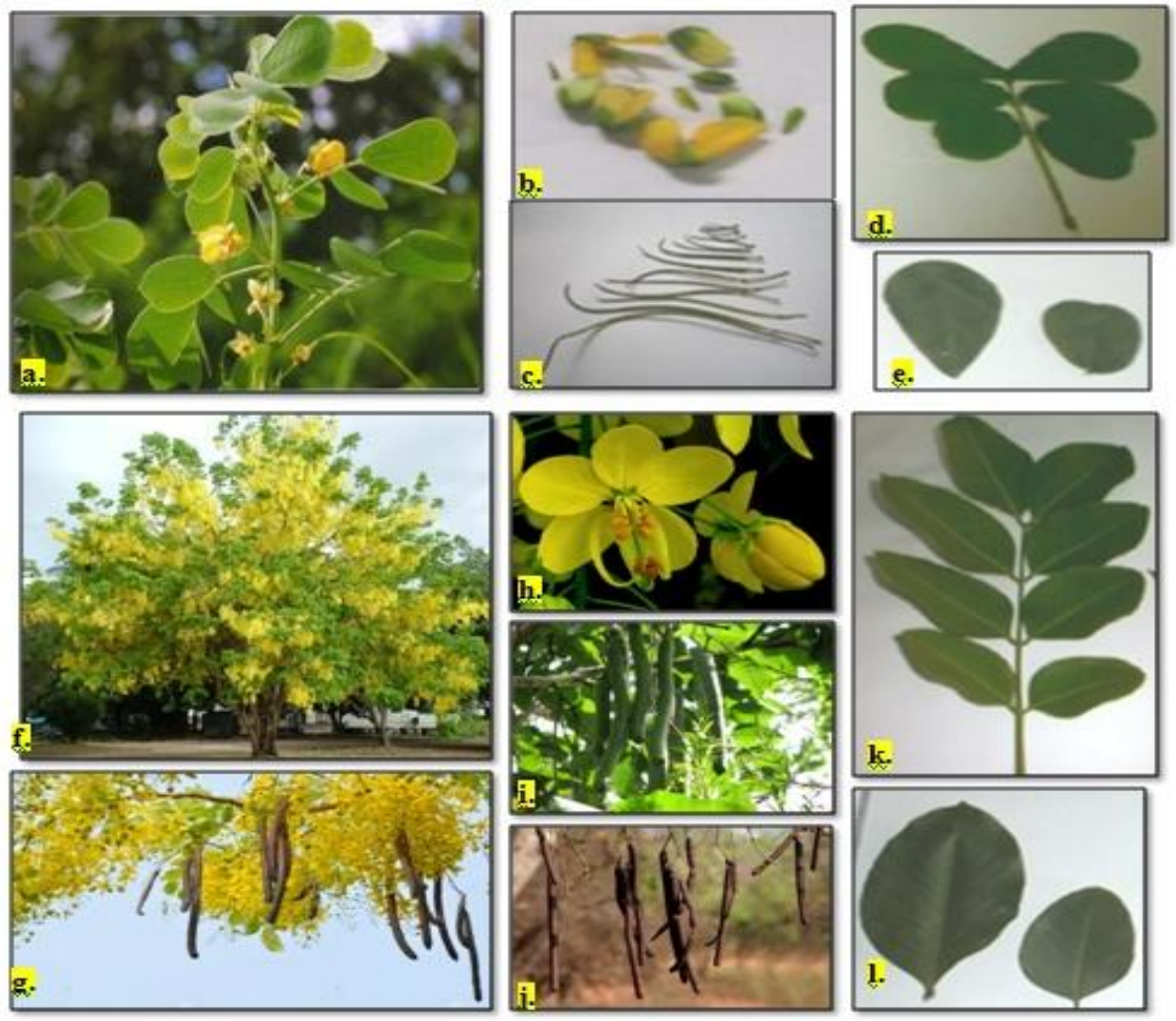
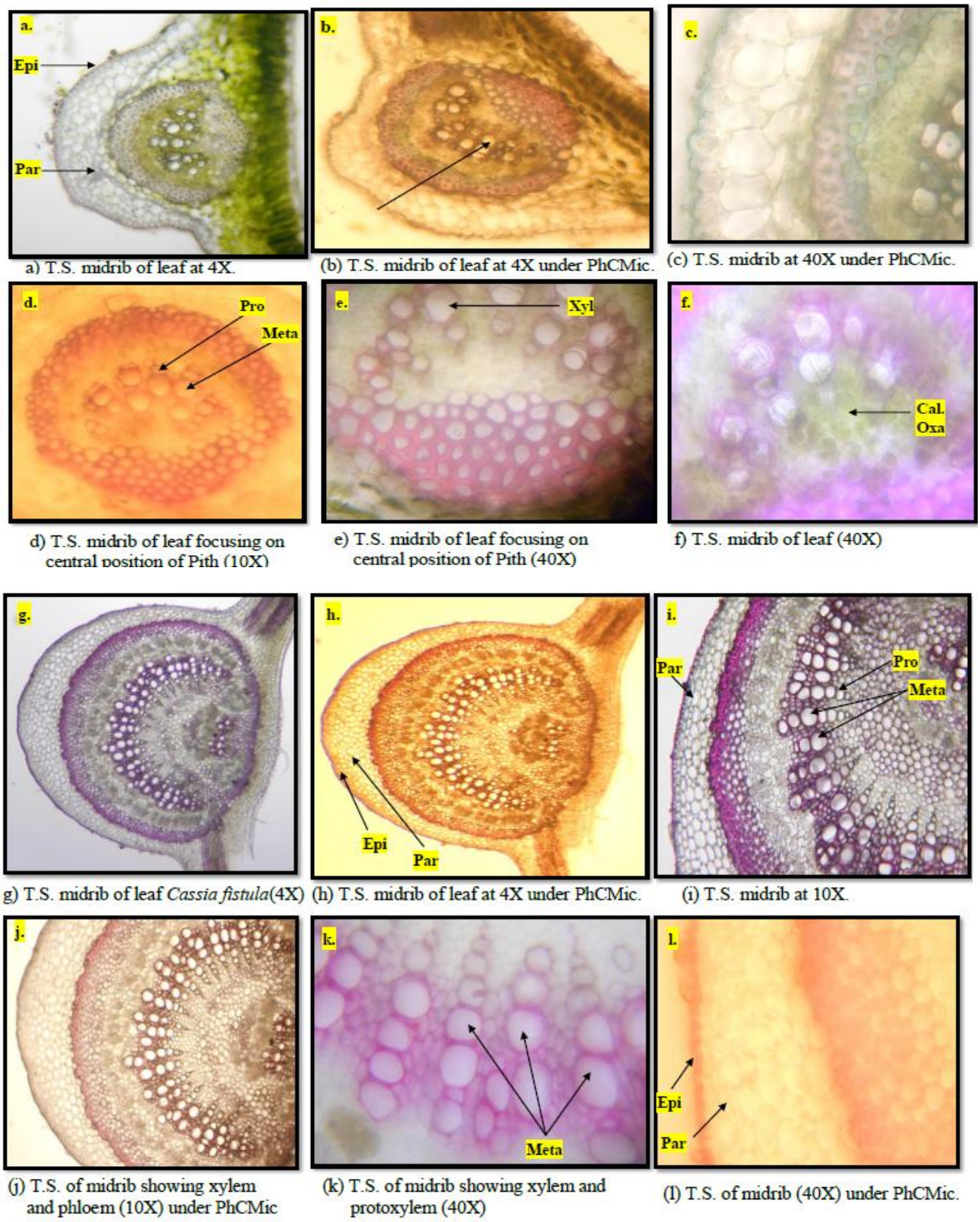


Fig. 1: Macromorphological photographs of *Cassia species.*

i) *Cassia tora* (a- e), a) An annual herb; b) Flower; c) Young pods d) Compound leaves e) Leaves (3-4 inches)

ii) *Cassia fistula* (f- l) f) Perinnial Tree; g) inflouresence with mature fruits; h) Flower; i)Young Pods; j) Mature Pods; k) Compound leaves l) leaves (9-16 inches)



a) T.S. midrib of leaf at 4X. (b) T.S. midrib of leaf at 4X under PhCMic. (c) T.S. midrib at 40X under PhCMic.

d) T.S. midrib of leaf focusing on central position of Pith (10X) e) T.S. midrib of leaf focusing on central position of Pith (40X) f) T.S. midrib of leaf (40X)

g) T.S. midrib of leaf *Cassia fistula*(4X) (h) T.S. midrib of leaf at 4X under PhCMic. (i) T.S. midrib at 10X.

(j) T.S. of midrib showing xylem and phloem (10X) under PhCMic (k) T.S. of midrib showing xylem and protoxylem (40X) (l) T.S. of midrib (40X) under PhCMic.

Key – PhCMic – Phase contrast Microscope ; Epi – Epidermis ; Xyl – Xylem ; Par – Parenchyma ; Pro – Protoxylem ; Met – Metaxylem; Cal. Oxa – Calcium Oxalate Crystals.

Fig.1B: Photo- Micrograph of leaves of *Cassia sp.*: I. *Cassia tora* (a- f), II. *Cassia fistula* (g- l)

Powder analysis

The powders of both the species were analyzed for organoleptic characters and differences were observed (Fig. 2 and Table 2).

I. (a.) *Cassia tora* II. (b.) *Cassia fistula*

Figure 2: Photographs: Powders of leaves of *Cassia sps.* (Organoleptic Characters)

Table 2. Organoleptic Characters

S. N.	Characters of Powder	Observation	
		Cassia tora	***Cassia fustula***
1	Colour	Dill green	Yellowish green
2	Odour	Mild Aromatic	Mild pleasuent aromatic
3	Taste	Slightly Bitter	Slightly bitter
4	Size	Course	Fine
5	Miscellaneous	Cellulosic fibers	Fibres

Physicochemical evaluation

The evaluation of physico-chemical parameters, was carried out to ensure quality and purity of the crude sample (Table – 3).

Foreign organic matter (FOM)

The matter or part of the matter other than the crude drug which is not defined and described in the prescribed monograph of sample is known as foreign organic matter. High percentage of foreign organic matter is considered as a more deteriorating quality of drug or sample (Mukherjee, 2002). The content of FOM in selected samples was found in appraisable limit *i.e.* 1.5% ± 0.005 and 1.0% ± 0.002 for *C. tora* and *C. fistula* respectively (Table 3).

Total moisture content (TMC)

Total moisture content (Loss on drying) measures the amount of water and volatile matters or minerals *viz.* Cu, Fe, Pb, Hg, Ni, Zn in a sample when the sample is dried under specified conditions. Loss on drying is the loss of mass expressed as w/w. In both the samples, TMC values were found in negligible percentage *i.e.* 0.7 % ± 0.002 in *C. tora* and 1.5 % ± 0.07 in *C. fistula*.

Ash values

Ash values (Total ash, Acid Insoluble ash, Water soluble ash and Sulphated ash) of a drug give a relevant reference of the earthy matter or the inorganic composition and other impurities present along with the drug. The Total ash gives the information of physiological (plant tissues) and non-physiological ash (external matter adhering to plant surface) content in organic matter. Crude sample of *C. tora* leaves exhibited higher total ash content (17.35 % ± 0.003) than *C. fistula* (10.3% ± 0.05). Acid insoluble ash measures presence of amount of silica or silicates in the form sand or siliceous earth and in both the leaves of *Cassia,* it was found in permissible limit with negligible difference *i.e.* 2.20% ± 0.02 and 2.50% ± 0.04 for *C. tora* and *C. fistula* respectively (Table 3). The Water soluble ash is useful to determine inorganic content of ash of crude drug which is found to be soluble in water as this gives a useful indication of the quality of plant material. The powder of the *C. fistula* leaves was exhibited higher water soluble ash content (13.00% ± 0.033) than *C. tora* (9.06% ± 0.002). The Sulphated ash test is an analytical test for determining the content of inorganic impurities or residual matter in an organic substance which is not volatilized from a sample when the sample is ignited in the sulfuric acid. Sulphuric acid reacts with inorganic compounds and converted into their sulfates and preferably metal oxides. Total Sulfated Ash (%w/w) was found to be higher in *C. tora* (15.60 % ± 0.03) than *C. fistula* (7.06% ± 0.002).

Extractive values

The extractive values are primarily useful for the determination of exhausted or adulterated drug. The alcohol extractive (A.E.) values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids, etc. and the water extractive (W.E) values indicated the

presence of sugar, acids and inorganic compounds (Indian Pharmacopoeia, 1996; Mukherjee, 2002). Both the extractive values [alcohol and water (%w/w)] were found to be maximum in *C. fistula* than in *C. tora* (Table-3). In both the plants, water extractive value was higher than alcohol extractive value. This signifies that the large amount of constituents of leaves were soluble in water than alcohol.

Heavy Metal analysis:

Contamination of medicinal plant material with heavy metals can be attributed to many causes including environmental pollution and traces of pesticides. Limit tests for such toxic metals are essential for herbal ingredients. In the examination of leaves of *C. tora* and *C. fistula*, all the selected heavy metals (Hg; As; Pb; Cr; Cd) were found to be less than 0.01ppm *i.e.* below permissible limit set by FAO/WHO for medicinal herbs and edible plants. Zinc (Zn) is also an essential trace element; plays an important role in various cell processes and WHO's recommended limit for Zn in medicinal plant is 50mg/kg *i.e*. 50ppm (Shah *et al.*, 2013; Jabeen *et al.*, 2010). In the leaves of *Cassia sps.*, Zn was found within the range of permissible limit *i.e*. in *C. tora* - 23.67 ppm and in *C. fistula* – 5.07 ppm.

Foaming and Swelling index

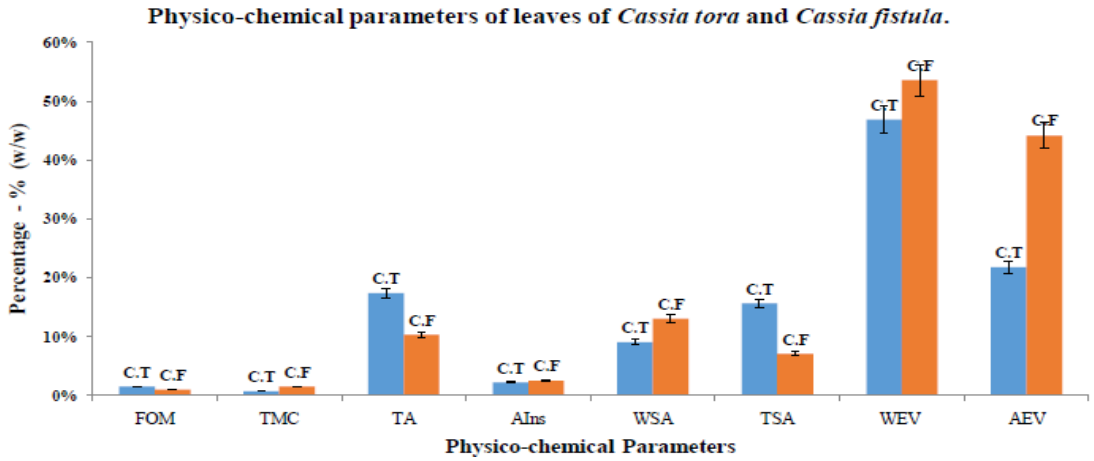
Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming index measured the foaming ability of an aqueous decoction of plant materials and their extracts due to presence of saponin. In both of the species, it was found to be less than 100 ml. Swelling index gives an idea of the mucilage content in the crude drug**.** Powder of *Cassia species i.e*. *C. tora* and *C. fistula,* leaves showed no swelling index (Table-3).

pH determination

pH refers to H^+ ion concentration in terms of acidity and basicity of the sample and its solvent. Leaves of *C. tora* and *C. fistula* extracts showed approximately same range of pH values *i.e.* 6 to 6.8 (Table-3).

Table 3: Physico-chemical parameters of leaves of *Cassia tora* and *Cassia fistula.*

S. No.	Parameters	*Cassia tora*	*Cassia fistula*
1.	Foreign organic matter **(%w/w)**	1.5% ± 0.005	1.0% ± 0.002
2.	Total Moisture Content **(%w/w)**	0.7 % ± 0.002	1.5 % ± 0.07
	Ash Values		
3.	Total Ash **(%w/w)**	17.35 % ± 0.003	10.3 % ± 0.05
4.	Acid Insoluble Ash **(%w/w)**	2.20 % ± 0.02	2.50 % ± 0.04
5.	Water soluble Ash **(%w/w)**	9.06 % ± 0.002	13.00 % ± 0.033
6.	Total Sulfated Ash **(%w/w)**	15.60 % ± 0.03	7.06 % ± 0.002
	Extractive Values		
7.	Water Extractive Value **(%w/w)**	46.88 % ± 0.05	53.6 % ± 0.2
8.	Alcohol Extractive Value **(%w/w)**	21.76 % ± 0.01	44.16 % ± 0.05
	Heavy Metal analysis (ppm)		
9.	Arsenic (As)	<0.01 ppm	<0.01 ppm
10.	Cadmium(Cd)	<0.01 ppm	<0.01 ppm
11.	Chromium(Cr)	<0.01 ppm	<0.01 ppm
12.	Lead (Pb)	<0.01 ppm	<0.01 ppm
13.	Mercury (Hg)	<0.01 ppm	<0.01 ppm
14.	Zinc (Zn)	23.67 ppm	5. 07 ppm
	Miscellaneous Parameters		
15.	Foaming index (ml)	< 100	< 100
16.	Swelling index	Nil	Nil
17.	pH (5 %)	6.5 ~ 6.8	6 ~ 6.8



Key:- C.T. *Cassia tora,* **C.F.:** *Cassia fistula;* **FOM:** Foreign organic matter, **TMC:** Total moisture contents **T.A.:** Total Ash; **AIns:** Acid insoluble Ash; **WAS:** Water soluble Ash; **TSA:** Total Sulfated Ash; **WEV:** Water Extractive Value; **AEV:** Alcohol Extractive Value

Fig. 3: Graphical representation of physicochemical parameters of *Cassia tora* and *Cassia fistula*

Table 4: Fluorescence Characters of the powdered leaves of *Cassia tora* and *Cassia fistula* under Ultra violet (UV) light

Sr. No.	Treatment	Fluorescence	
		C. tora	*C. fistula*
1	Powder mounted with nitrocellulose .	Greyish white	Greyish white
2	Powder treated with NaOH in methanol.	Green	Greenish Black
3	Powder treated with NaOH in water.	Yellow	Violet
4	Powder treated with NaOH in water dried and mounted with nitro cellulose.	Brown	Greenish Red
5	Powder treated with NaOH in methanol dried and mounted with nitro cellulose.	Yellowish green	Yellowish green
6	Powder treated with HCl	Grey	Bluish Black
7	Powder treated with HCl dried and mounted with nitro cellulose.	Greenish Yellow	Greenish Yellow
8	Powder treated with HNO_3 diluted with equal volume of water.	Blue	Grey

Table 5: Phytochemical screening of *Cassia tora* and *Cassia fistula* leaves

SOLVENTS	E.A		Chloroform		Acetone		P.E		Methanol		Aqueous	
Phytoconstituents	C.T	C.F	C.T	C.F	C.T	C.F	C.T	C.F	C.T	C.F	C.T	C.F
Alkaloids	–	+	–	–	–	+	+	+	+	+	+	+
Flavonoids	–	+	+	+	–	–	+	+	+	+	+	+
Glycosides	+	+	+	+	–	–	+	–	–	+	+	–
Anthraquinone	–	–	–	–	+	–	–	–	+	+	+	–
Phenols	–	+	–	–	–	+	+	+	+	–	+	+
Saponins	–	–	–	–	–	–	–	–	–	+	+	+
Steroids	+	+	+	–	+	-	+	–	+	–	+	–
Triterpenoids	–	+	–	–	–	+	–	+	–	+	+	–
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	+	–	+	–	+	+	+	–	+	+	+	+
Proteins	–	+	–	–	–	-	–	–	+	+	+	+

Key: C.T : *Cassia tora*; C.R : *Cassia fistula* ; E.A : Ethyl Acetate; P.E : Petroleum ether.
'+' = Detected ; '–' = Not Detected.

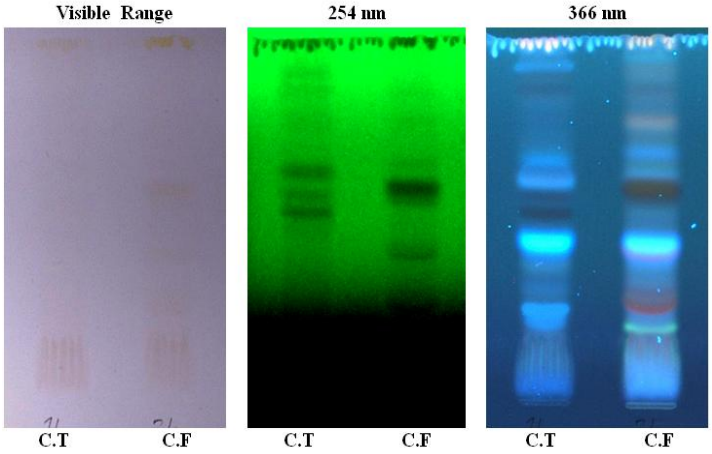


Fig. 4: Thin layer Chromatography of *Cassia sp.*

Fluorescence powder drug analysis:

Fluorescence analysis of powdered drug is another distinguishing parameter for identification of crude sample. When powdered drug and extracts were treated with different reagents, colour of the crude powder changed and when observed under UV light, they emitted various colored radiations or fluorescence (Table 4).

Preliminary phytochemical screening:

The preliminary phytochemical investigations of ethyl acetate, chloroform, acetone, petroleum ether, methanol and aqueous extracts of *Cassia tora* and *Cassia fistula* leaves were performed (Table-5). Maximum phyto-constituents were found in methanol and aqueous extracts of leaves of *C. tora* and *C. fistula* and showed the prominent presence of major secondary metabolites like flavonoids, tannins, phenolic compounds, anthraquinone, triterpenes and carbohydrates. Whereas non-polar solvent extracts such as ethyl acetate, chloroform, acetone and petroleum ether showed minimum phytocompounds. The presence of various phytoconstituents may help to develop therapeutic activity of both the species of *Cassia*.

TLC finger printing profile:

The TLC profile of hydro-alcoholic extracts of *Cassia sps.* leaves was established and carried out in solvent system [Ethyl Acetate : Formic Acid : Glacial Acetic Acid : Water (Wagner *et al.;* 2007)]**.** In developed TLC plates, number of prominent bands were observed at short (254 nm) and long (366 nm) UV wavelength, indicating the presence of various types active phyto-compounds in sample. TLC plates were further derivatized for confirmation of phytocontituents (Fig. 4).

CONCLUSION

Standardization is an essential measurement for ensuring the quality control of any herbal drugs. The pharmacognostic features (morphology, microscopy), physicochemical constants (foreign organic matter, total moisture, ash values, extractive values, heavy metals quantification, index values, pH etc.) and phytochemical screening are some of the integral parameters for the standardization of any crude or herbal drug. The pharmacognostical evaluation and physico-chemical parameters, may help in differentiation of *Cassia sps. viz. Cassia tora* and *Cassia fistula*, based on their morphology, anatomy and physico-chemical characters. Phytochemical screening gave qualitative data of various phytoconstituents that further can be harvested for characterization of bioactive compounds. All the above information may act as reference for correct identification and authentication of plant materials (*C. tora* and *C. fistula*). Moreover, these

investigations will help in standardization and evaluation of quality (*i.e.* impurities and adulterant identification) of crude sample which further can be useful in formulation of herbal or medicinal drug. Thus the present comparative study is significant for standardization and quality assessment of crude drug.

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RESEARCH ARTICLE

Comparative study of young and mature leaves of *Terminalia catappa* for evaluation of Physico-chemical, Pharmacognostical and Phytochemical analysis

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Abstract

In recent years there has been rapid increase in the standardization of selected medicinal plant of potential therapeutic significance. Despite the modern techniques, identification of plant drug by pharmacognostic study is more reliable. *Terminalia catappa* belongs to family Combretaceae, commonly known as Indian Almond, native to most regions of the India. By looking at the high traditional use of the young and mature leaves of plant *Terminalia catappa* Linn, the present investigation was undertaken for research with the purpose of drawing the pharmacopoeial standards for this species. The present study deals with pharmacognostical parameters for the young and mature leaves of *Terminalia catappa* which mainly consists of macroscopic and microscopic characters, powder characteristics, physio-chemical constants and phytochemical screening. The macroscopic, microscopic characteristics of Almond leaves were identified. In physico-chemical parameters, foreign matters, total moisture content, pH, different ash contents [total ash, acid insoluble ash, water soluble ash, sulphated ash] and extractive values [alcohol soluble and water soluble] were studied. In qualitative phytochemical analysis, it was observed that the mature leaves of *Terminalia catappa* contain more phytoconstituents as compared to young leaves. The study revealed specific characteristics for the particular crude drug which will be of significant use in identification, control to adulterations of raw drug and can serve as a reference for any further investigations.

Keywords: *Terminalia catappa*, Physicochemical study, Pharmacognostic study, Phytochemical screening.

INTRODUCTION

Medicinal plants are plants that have at least one of their parts (leaves, stem, barks or roots) used for therapeutic purposes (Bruneton, 1993). Recently, medicinal plants have become important for the treatment of different disease conditions such as diabetes, malaria, anaemia (Fola, 1993). The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor *et al.*, 2005). The importance of medicinal plants, and the contribution of phytomedicines to the well-being of a significant number of the world's population, has attracted interest from a variety of disciplines. It is no wonder that the world's one-fourth population i.e. 1.42 billion people, are dependent on traditional medicines for the treatment of various ailments. However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control (Jena *et al.*, 2011). There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as a medicine. The process of standardization can be achieved by stepwise pharmacognostic and phytochemical studies. These studies help in identification and standardization of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. *Terminalia catappa* Linn. (Indian almond) commonly known as Laal Badam (Red almond), belonging to Combretaceae family. It grows best in moist tropical climates. The species loses its leaves twice a year in most areas, with a brilliant red-and-yellow display of leaf-fall. Although Indian almond does grow when planted on uplands, the natural habitat of the species is in areas just inland from ocean beaches, near river mouths and on coastal plains. These areas are typically flat, but they may have dunes or rocky bluffs (Orwa *et al.*, 2009). In the traditional Indian system of medicine, the ayurveda and various folk system of medicine, *Terminalia catappa* possess several medicinal properties. It is very rich in phytochemicals and a good source of natural antioxidants (Punniya *et al.*, 2014). Parts of the tree, such as the leaves and fruit, are astringent. The leaves, crushed with *Dacrydium elatum* and rhizomes of *Cyperus rotundus*, are combined to treat dysentery. The red leaves act as a vermifuge, while the sap of young leaves, cooked with oil from the kernel, is used to treat leprosy. Leaves, bark and fruit are used to treat yaws. The bark and root bark are useful for bilious fever, diarrhoea, thrush, and as a remedy for sores and abscesses. The kernel of the fruit mixed with beeswax stops putrid exudation and bloody faeces. It is recommended as a mild laxative and a galactagogue for women, but too frequent use causes diarrhoea. The young leaves are used to cure headaches and colic (Arumugam *et al.*, 2015). In the present work pharmacogno- stical, physicochemical and phytochemical characteristics were studied on young and mature leaves of *Terminalia catappa*.

MATERIAL AND METHODS

***Material*:** Young (Green) and mature (red) leaves of *Terminalia catappa* (Indian almond) were collected in the month of September 2014 from Thakurli near Kalyan of Maharashtra, India. The plant was identified and authenticated from the Blatter Herbarium, St. Xavier's College, Mumbai. All chemicals used in assays were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck Co. (Santa Ana, CA, USA).

Preparation of the extracts and fractions:

The air-dried powdered young (green) and mature (red) leaves of *T. catappa* (5 g) were extracted with n-butanol, chloroform, acetone, petroleum ether and water using a mechanical shaker for 12-18h. The resultant extracts were concentrated using sonicator. Then the crude n-

butanol, chloroform, acetone, petroleum ether and aqueous extracts were filtered and fractions were used for further analysis.

***Macroscopic Description*:** The *Terminalia catappa* was subjected to macroscopic studies which comprised of organoleptic characteristics *viz*. color, odour, taste, size, etc. of the drug. These parameters are considered as useful in quality control of the crude drug and were evaluated as per standard WHO guidelines (Khandelwal, 2006; Kokate, 1997; Wallis, 2005).

***Microscopic Description*:** Free hand transverse sections of the leaves were taken, stained with safranin and fast green. Then observed under compound microscope (Lawrence & Mayo–LM–52-1602) and Phase contrast microscope (Lawrence & Mayo–LM–52-1802) for their peculiar characters.

***Powder characteristics*:** Preliminary examination of the young and mature leaf powder with different chemical reagents and microscopical observation was carried out as per reported methods (Iyengar and Nayak, 2008; Iyengar 1997). The powder characteristics were observed using compound and Phase contrast microscope.

***Physicochemical Evaluation*:** Analysis of physicochemical constants of the young and mature leaf powder has been done to evaluate the quality and purity of the drug. Various physic-chemical parameters like moisture contents, foreign organic matters, pH, ash values and extractive values were calculated as per WHO guidelines. The information collected from these test will be useful for standardization and obtaining the quality standards (Indian Pharmacopoeia, 1996; World Health Organization 1998).

***Preliminary phytochemical analysis*:**
The qualitative chemical tests were carried out for the identification of the different phytoconstituents present in the powered crude drug. (Saxena *et al.*, 2012). Presence of Alkaloids, Flavonoids, Cardiac glycosides, Anthronal glycosides, Phenols, Saponins, Sterols, Triterpenoids, Tannins, Hydrolysable Tannins, Carbohydrate, Starch and Proteins were tested by using standard qualitative methods.

RESULTS AND DISCUSSION

Macroscopic description

Terminalia catappa is a tall deciduous and erect tree reaching 15-25 m, trunk 1-1.5 m in diameter, often buttressed at the base. Whorls of nearly horizontal, slightly ascending branches spaced 1-2 m apart in tiers or storeys are present up the trunk. The pagoda-like habit becomes less noticeable as the branches elongate and droop at the tips. Bark grey-brown, rough with age. The leaves of *Terminalia catappa are* alternate obovate with short petioles, spirally clustered at the branch tips, 15-36 cm long, 8-24 cm wide, dark green above, paler beneath, leathery and glossy. They turn bright scarlet, dark red, dark purplish-red, or yellow (Fig. 1 and Table 1).

The organoleptic evaluation of the young and mature leaves of *T. catappa* powder revealed that young leaves powder was grayish green in color with mild pleasant aromatic odour, agriable taste whereas mature leaves powder was brownish red colour, having mild earthy odour and was tasteless (Table 2).

Microscopic Description

The T.S. of midrib (Fig. 3a, 3b, 3c) showed dorsiventral structure and a distinct biconvex out line in the basal region where as in the apical region it is Plano convex. The T.S. showed single layered epidermis covered with thick cuticle. Epidermal cells of the ventral side and dorsal side were rectangular in shape with distinct thickening on radial walls. Some of the epidermal cells on the ventral sides elongated to form covering of pointed trichomes. Beneath the epidermal cells on either side the layers of collenchymatous cells were present however towards ventral side they are wider with 3–4

layers of cells. Followed by collenchyma cells, the 6–7 layers of parenchymatous cells with angular thickening were observed (Fig. 2a). Some of the parenchyma cells showed presence of rosette of calcium oxalate crystal (Fig. 4b). Arc-shaped/ Triangular vascular strand showed layer of xylem surrounded by phloem on either side of xylem. Xylem consisted of metaxylem and protoxylem. Metaxylem showed extended arms of protoxylem on both the sides (Fig. 4a). 3 Air cavities were observed in the ring of vascular strand with presence of giant crystal located in the pith region (Fig. 4c). Hairs were extended from the epidermal layer of cells (Fig. 4d). In mature leaves a prominent layer of lignified cells was observed in the apical region of the midrib surrounding the vascular tissue (Fig. 2, 3b).

Table 1: Characters of leaf of *Terminalia catappa*

Sr. No.	Characteristic features	Description
1	Arrangement	Alternate
2	Type	Simple
3	Margin	Entire
4	Shape	Obovate
5	Length	8-12 Inches
6	Colour	Green
7	Fall colour	Red
8	Fall characteristic	Showy

Table 2: Organoleptic characters of *Terminalia catappa.*

Sr. No.	Characters	Observation	
		Young leaves	Mature leaves
1	Colour	Grayish green	Brownish red
2	Odour	Mild pleasant aromatic	Mild earthy
3	Taste	Agriable	Tasteless
4	Size	Course	Medium
5	Miscellaneous	Cellulosic fibers	Fibers

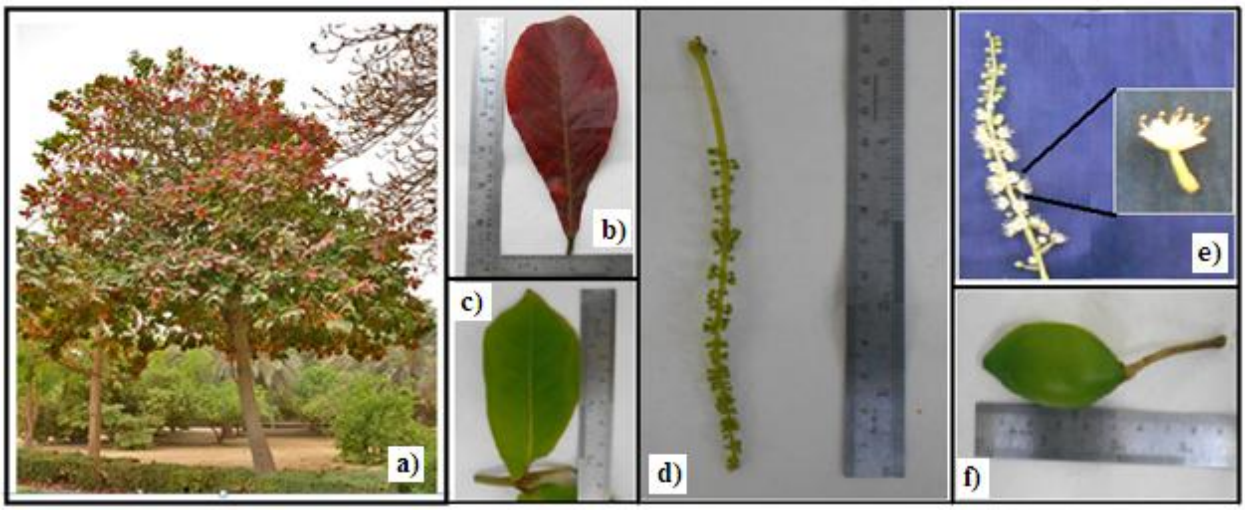

Fig. 1: Macroscopic description – a) *Terminalia catappa* Tree; b) Mature leaf of *T. catappa;* c) Young leaf of *T. catappa;* d) Influorensence; e) Flower of *T. catappa;* f) Fruit of *T. catappa.*

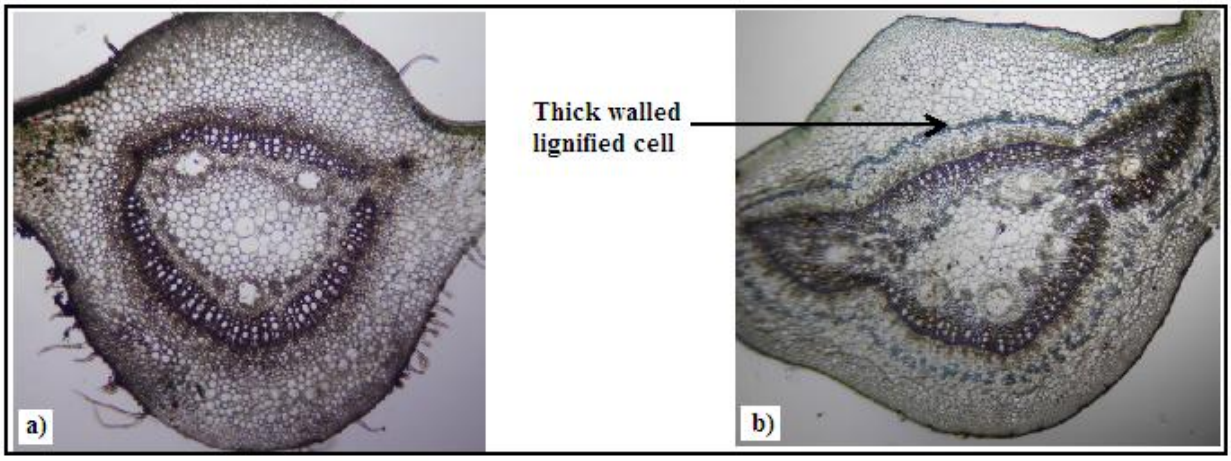

Fig. 2: (a) T.S. of young leaf *Terminalia catappa* through midrib at 4X (b) T.S. of mature leaf *Terminalia catappa* through midrib at 4X

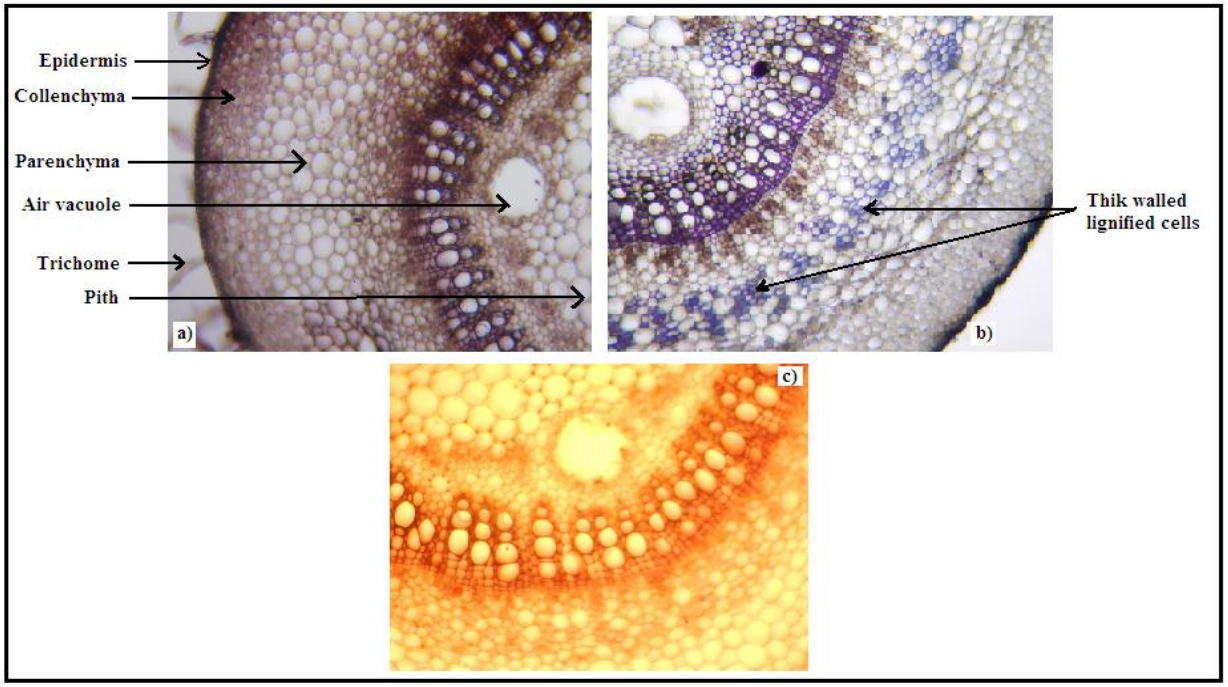

Fig. 3: (a) T.S. of leaf through midrib at 10X, (b) T.S. midrib of mature leaf showing thick walled lignified cells at 10X (c) T.S. of midrib under phase contrast microscope at 10X

Table 3: Ash values for young and mature leaves of *Terminalia catappa.*

Parameters	Leaves of *Terminalia catappa*	
	Young	Mature
Foreign matter (% w/w)	1.23% ± 0.001	1% ± 0.001
Total Moisture Content (% w/w)	5.71% ±0.002	4.32% ± 0.002
Total Ash (% w/w)	8.89% ±0.004	8.60% ± 0.004
Acid Insoluble Ash (% w/w)	4% ± 0.002	1.71% ± 0.001
Water soluble Ash (% w/w)	4.71% ± 0.002	2.51% ± 0.001
Sulfated Ash (% w/w)	9.80% ± 0.001	10.40% ± 0.003
pH (5%)	3.0 - 5.5	3.5 - 5.5

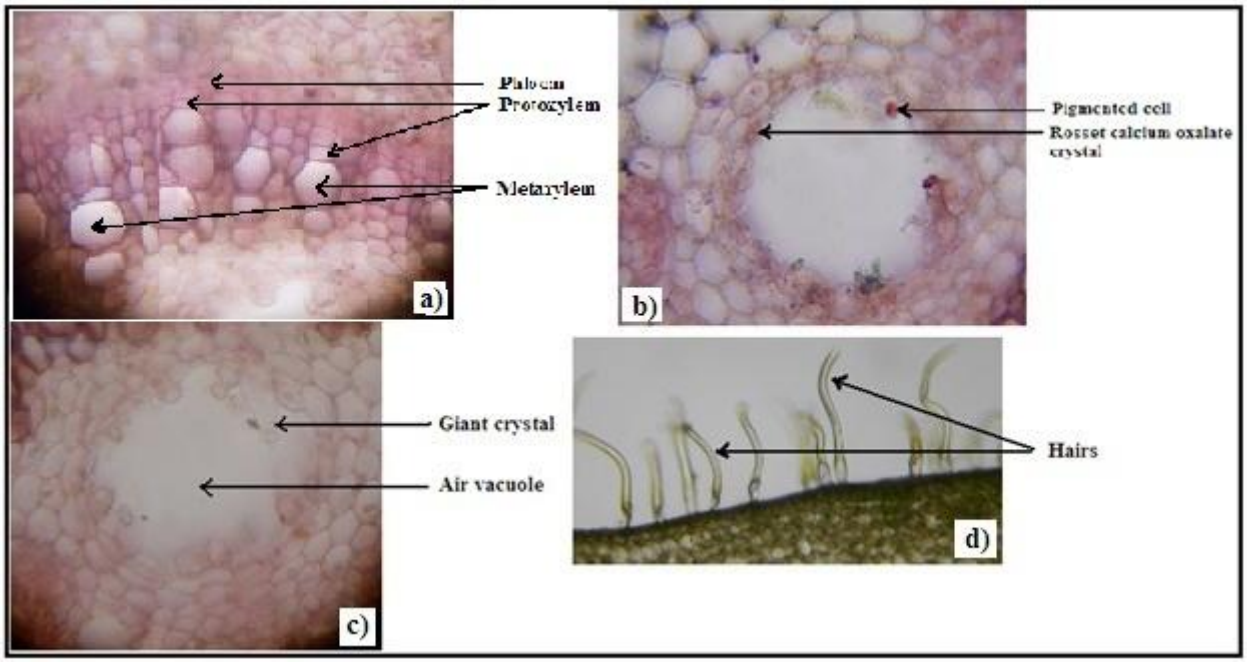

Fig. 4: (a) T.S. of leaf midrib at 40X showing xylem and phloem (b) T.S. of midrib showing pigmented cell and rosset calcium oxalate crystal at 40X (c) T.S. of midrib showing giant crystal (d) T.S of midrib showing hairs at 40X

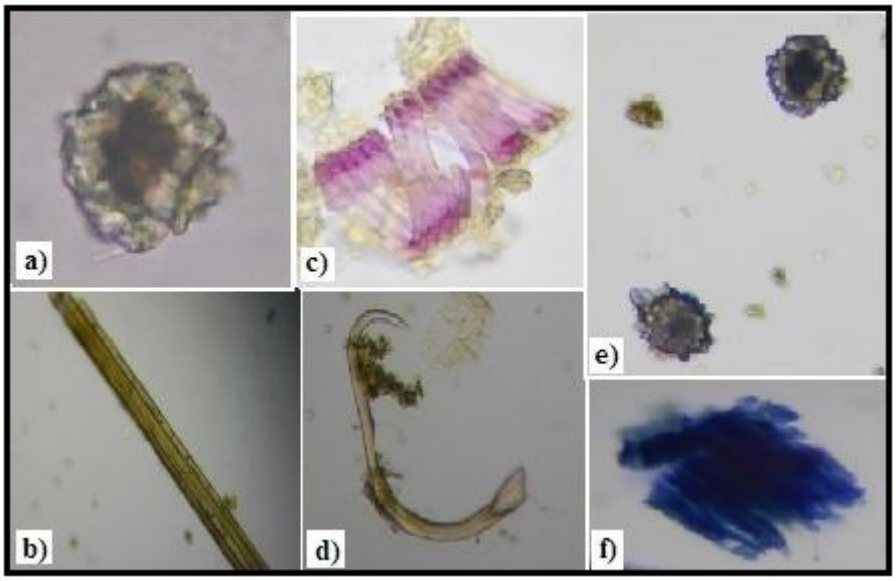

Fig. 5: Microscopy of leaf powder a) rosette calcium oxalate crystals (40X) b) phloem fibres (10X), c) xylem vessel (10X), d) unicellular trichome (10X) e) rosette calcium oxalate crystals (10X), f) cellulose (40X).

Table 4: Extractive values for young and mature leaves of *Terminalia catappa.*

Parameters	Leaves of *Terminalia catappa*	
	Young	**Mature**
Water Extractive Value (**% w/w**)	7.55% ± 0.002	10.45% ± 0.001
Alcohol Extractive Value (**% w/w**)	10.27% ± 0.002	11.97% ± 0.002

Table 5: Phytochemical screening of young and mature leaves of *Terminalia catappa.*

Solvents	n-butanol		Chloroform		Acetone		P. Ether		Aqueous	
Phytoconstituents	**TG**	**TR**	**TG**	**TR**	**TG**	**TR**	**TG**	**TR**	**TG**	**TR**
Alkaloids	-	-	-	-	+	+	-	+	-	+
Flavonoids	-	+	-	-	+	+	-	-	-	+
Cardiac glycosides	+	+	+	+	-	-	+	+	-	-
Anthronal glycosides	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	-	-	-	+	-	-	+	+
Saponins	-	-	-	-	-	-	-	-	-	-
Sterols	+	+	+	-	+	-	+	-	-	-
Triterpenoids	+	+	-	+	-	+	-	+	+	+
Tannins	-	+	-	-	-	+	+	+	-	+
Hydrolysable tannins	-	+	-	-	+	+	-	-	-	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+
Starch	-	-	-	-	-	-	-	-	-	-
Proteins	-	-	-	-	-	-	-	-	-	-

Key : TG: Young green leaves of *T. catappa*, **TR:** Mature red leaves of *T. catappa* **P. Ether**: Petroleum Ether ; " **+**" Detected ; "**–**" Not Detected.

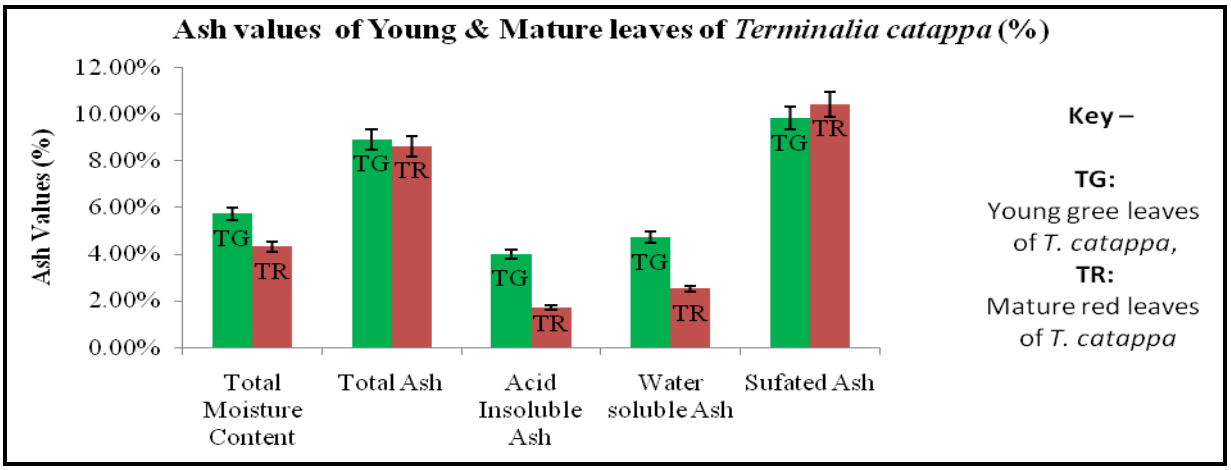

Fig. 6: Graphical representation of mean ash values of young and mature leaves of *Terminalia catappa*

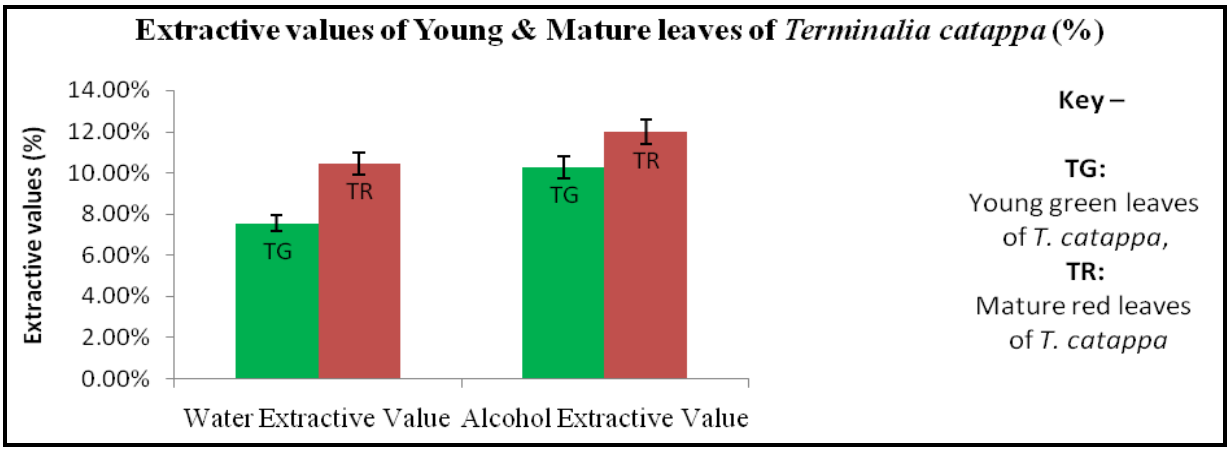

Fig.7: Graphical representation of mean extractive values of young and mature leaves of *Terminalia catappa.*

Powder Characteristics

The powder of young leaves of *Terminalia catappa* was grayish green in color with mild pleasant aromatic odour, agriable taste whereas mature leaf powder was brownish red in colour, having mild earthy odour and was tasteless. The microscopic examination of the powder of young and mature leaves showed similar characteristic features like presence of rosette calcium oxalate crystals, phloem fibres, xylem vessel, unicellular trichome and cellulose (Fig. 5).

Physicochemical Evaluation:

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The foreign organic matter give the presence of any organism, part or product of an organism, other than that named in the specification and description of the herbal material concerned in Indian Pharmacopoeia, 1996 (Mukherjee, 2002). The foreign organic matter was found to be 1.23% ± 0.001 and 1% ± 0.001 for young and mature leaves respectively, it indicated that their may be presence of part or product of an organism in very less amount. The total ash value was higher than that of the acid insoluble and water soluble ash value for both young and mature leaves and a decrease in the acid insoluble ash value may be due to presence of smaller quantity of silicaceous matters. The extractive values are primarily useful for the determination of exhausted or adulterated drug. The water extractive value indicates the presence of sugar, acids and inorganic compound (Indian Pharmacopoeia, 1996, Mukherjee, 2002). The water extractive value was found to be 7.55% ± 0.002 and 10.45% ± 0.001 for young and mature leaves respectively (Table 5). The alcohol extractive value indicates the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. (Indian Pharmacopoeia, 1996, World Health Organization, 1998). The alcohol extractive value was found to be 10.27% ± 0.002 and 11.97% ± 0.002 for young and mature leaves respectively (Table 5). This signifies that the large amount of constituents of leaves were soluble in alcohol than water.

Preliminary Phytochemical Screening

The preliminary phytochemical investigations of n-butanol, chloroform, acetone, petroleum ether and aqueous extracts of young and mature leaves of *Terminalia catappa* were performed. The young leaves of *Terminalia catappa* showed the presence of Phenolic compound, Glycosides, Sterols, Saponins, Triterpenes and Carbohydrate type of major secondary metabolites whereas mature leaves of *Terminalia catappa* showed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, triterpenes, tannin and carbohydrate as major secondary metabolites which revealed their potent therapeutic activity. In young leaves of *Terminalia catappa*, n-butanol and acetone extracts showed presence of higher phytochemicals whereas in mature leaves of *Terminalia catappa*, n-butanol extract showed presence of higher phytochemicals. The results showed that, the mature leaves of *T. catappa* contain more amounts of phytochemicals as compared to young leaves, so it can be concluded that with the increasing age of leaves, there is also increase in the amount of phytoconstituents.

CONCLUSION

Standardization is essential measure for quality, purity and sample identification. Macroscopy and microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. Studies of physicochemical constants can serve as a valuable source of information and are usually used in judging the purity and quality of the drug. The extractive values give an idea about the maximum extraction of drug in particular solvent and from the study, for both young and mature leaves, the extractive value of alcohol was highest followed by water. The mature leaves of *Terminalia catappa* showed presence of higher

phytoconstituents than young leaves. The preparations made from the leaves of *Terminalia catappa* are currently being used in several traditional and folklore systems of medicine for the treatment of various diseases without standardization. These findings would help as a tool for characterization of *Terminalia catappa* with its pharmacognostic and physicochemical characteristics, discriminating it from its other species diversity and aid in further screening of young and mature leaves for anti-cancerous activity, fungicidal, anti-diabetic activity and other specific bioassays.

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RESEARCH ARTICLE

Evaluation of indole acetic acid and phosphate solubilisation by plant growth promoting Rhizobacteria (PGPR): Potential use as Biofertilizer

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers. A total of six plant growth promoting rhizobacteria (PGPR), designated as PGPR1, PGPR2, PGPR3, PGPR4, PGPR5, PGPR6 were isolated from the rhizosphere soil and were screened for production of indole acetic acid (IAA) and inorganic phosphate solubilisation. The isolate (PGPR6) showing both the activities was selected and characterised. The isolate was identified as *Pseudomonas fluorescence*. The amount of IAA produced and phosphate solubilised by PGPR6was estimated by standard methods. PGPR6produced 20mcg/ml of IAA and released 147mcg/ml of solubilised phosphate. The effect of varying concentrations of L-tryptophan and tricalcium phosphate on plant growth promoting activities of PGPR6 was studied on mung (*Vignaradiata*) seedlings. The optimum amount of L-tryptophan required by the isolate was found to be 5mg/ml. It was found that 0.5% is the optimum concentration of tricalcium phosphate required by the organism to support root development. The shoot length, root length, wet and dry weight of the seedlings were measured and recorded. The data was represented in graphical form. Pot experiment was conducted to evaluate the effect of PGPR6on vigour index of plant. The vigour index of 'TEST' seed inoculated with PGPR6 showed higher value (1654) as compared to 'CONTROL' (521.05). Hence, *Pseudomonas fluorescence* is a potential PGPR which can be used as a biofertiliser.

Keywords : IAA, phosphate solubilisation, PGPR, biofertiliser

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonise plant roots and benefit plants by providing growth promotion, directly or indirectly, The common traits include production of plant growth regulators (auxin, gibberellin, ethylene etc.), siderophores, HCN, antibiotics and inorganic phosphate solubilisation. Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoots growth. There has been an increasing number of PGPR being commercialized for various crops.

Pseudomonas fluorescence is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR. They are gram negative, motile, rod shaped bacteria and have diverse phytobeneficial traits. Their plant growth promoting activities include production of HCN, siderophores, protease, antimicrobials, plant growth promoting hormones such as IAA, phosphate solubilizing enzyme. Hence, they plays an effective role in stimulating yield and growth traits of crops and provides significant increases in fresh and dry masses (Rekha *et al*., 2010).

IAA is one of the most physiologically active auxins, produced by L- tryptophan metabolism by several microorganisms including PGPR (Sadaf *et al*., 2009). Effects of plant growth regulators including IAA on the plant will be concentration dependent. Farah etal., 2004 screened local isolates of *Pseudomonas sp.* for their intrinsic ability to produce IAA in the presence of varying amounts of L-tryptophan and their effect on root elongation of germinating seeds of test plants. Solubilisation of inorganic phosphate is another major phytobeneficial trait of *Pseudomonas fluorescence.* It is generally accepted that the mechanism of mineral phosphate solubilization by phosphate solubilising bacterial strains is associated with the release of low molecular weight organic acids, which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Chen *et al*., 2005). PGPR can improve the nutrient use efficiency of fertilizers and allow reduced application rates of chemical fertilizers. The use of PGPR isolates as inoculants biofertilizers is beneficial for crop cultivation as they enhance growth of crop and by inducing other plant growth promoting traits. Applying PGPR as biofertilizer affects beneficially the yield and growth of crop plants in field conditions (Yildirim *et al*., 2009, Subramaniam Gopala-krishnan *et al*. 2015). Given the negative environmental impacts of chemical fertilizers and their increasing costs, the use of PGPR is advantageous in the sustainable agricultural practices.

MATERIALS AND METHODS

Isolation of PGPR

Soil sample was collected from the rhizosphere of Country borage (*Coleus aromaticus*). The isolation of PGPR was done on Luria Bertani agar and King's B agar according to the method described by (Ashrafuzzman *et al*., 2009). The plates were incubated at 28±2°C for 48 hours. Shape, size, elevation, surface, margin, color and pigmentation etc. were recorded. Well isolated bacterial isolates were streaked onto fresh slants for maintenance and further characterization. The King's B agar plates were exposed to long wavelength UV light (365nm) for few seconds and the colonies exhibiting the fluorescence were picked up and streaked on to fresh King's B slants.

Screening for IAA production

Pure colonies of each isolate were grown in 100ml flasks containing 25ml of specific medium with and without L- tryptophan (5mg/ml). The screening for IAA was done according to method described by (Janardhan *et al*., 2010). Development of pink colour indicates IAA. The light absorbance was measured spectroscopically at 530nm.Amount of IAA produced was calculated

using the standard curve prepared with known concentration of IAA (Suresh *et al*., 2010).

Screening for phosphate solubilisation

Solubilisation of phosphate was determined by spot inoculation ofeach bacterial isolate on Pikovskayaagar medium (Pikovaskaya, 1948) (one isolate per plate) and incubated at 28 ± 2°C for 5 days and measuring the clear/halo zone around the isolate. Quantitative estimation of phosphate was carried out following ammonium molybdate-ascorbic acid method as described (Knudsen and Beegle, 1988).

Identification of PGPR isolate

Isolate showing positive for both IAA and phosphate solubilisation was identified by morphological and biochemical characterisation as described in Bergey's Manual of Determinative Bacteriology.

Seed germination test

Mung bean seeds were washed thoroughly under tap water and surface sterilized (Karnwal, 2009). The seeds were soaked in 18 hour old bacterial culture adjusted to 0.1 OD at 530nm (10^6cfu/ml) for 24 hours. The seeds were then transferred to water agar for germination at (28±2°C) for 24hours.

Effect of varying concentrations of L-tryptophan on seedling growth (Tryptophan optimization)

Jensson's seedling agar butts were prepared. Varying concentrations of L-tryptophan (1, 3, 5, 7, 9 mg/ml) were added to the butts aseptically (Farah *et al*., 2004). The bacteria coated seeds were transferred to butts aseptically (one seed per butt). Seed without bacterial coating was taken as control. The test tubes were cotton plugged and the lower portion of test tube upto the surface of butt was covered with aluminium foil to provide dark condition for seed germination. The agar butts were incubated for 5 days at room temperature (28±2°C) where sunlight is available. On the fifth day, seedlingswere removed without disturbing the root system and fresh weight of plant and dry weight of biomass were taken after drying samples to a constant weight in an oven (Fig3). The root length, shoot length were measured and recorded (Table1) (Ashrafuzzman *et al*., 2009). For studying the effect of varying concentrations of tricalcium phosphate on seedling growth, all the above procedures were kept same except that varying concentrations of tricalcium phosphate (0.3, 0.4, 0.5, 0.6, 0.7%) were used.

Pot experiment using PGPR isolate

Mung seeds were surface sterilized by method mentioned above. The seeds were soaked in 10 ml of bacterial suspension (10^6cfu/ ml) for 24 hrs and sterile blank nutrient broth served as control. Then the seeds were blot dried and sown in pots containing sterilized soil. The germination percentage was estimated at 10 days after sowing. Without disturbing the root system, the mung seedlings were depotted and observations on root length, shoot length, fresh weight of plant and dry weight of biomass were taken after drying samples to a constant weight in an oven and vigour index (VI) calculated (Dang, 2008).

VI = percent germination × mean total length of seedling (root length +shoot length)

RESULT

Isolation of PGPR

Six PGPR isolates were successfully isolated from the rhizosphere soil of Country borage (*Coleus aromaticus*). Five bacterial isolates were obtained from Luria Bertani agar medium. They were designated as PGPR1, PGPR2, PGPR3, PGPR4, PGPR5. One bacterial isolate was obtained on King's B agar medium. This isolate was designated as PGPR6 (Fig1).

Screening for IAA production

IAA production by PGPR isolates were analyzed. All six isolates showed positive for IAA production. PGPR6 was good producer of IAA whereas others showed poor IAA production. The

amount of IAA produced by PGPR6 was found to be 20mcg/ml.

Screening for phosphate solubilisation

Only PGPR6 showed the ability to solubilise phosphate. It produced a clear halo zone around the bacterial colony measuring about 3mm in diameter (Fig2). The amount of phosphate solubilised by PGPR6 was found to be 147mcg/ml.

Identification of PGPR isolate

PGPR6 showed positive for both IAA production and phosphate solubilisation. Hence it was selected for further characterisation. PGPR6 was identified as *Pseudomonas fluorescence.*

Effect of varying concentrations of tryptophan on seedling growth (Tryptophan optimization)

Highest seedling growth (15.3 cm) was observed at 5mg/ml tryptophan concentration followed by 7mg/ml tryptophan. However a decreased growth was shown at 9mg/ml tryptophan concentration, indicating that very high concentration of tryptophan may have a negative effect on seedling growth. Very poor growth was shown by seedlings at 1mg/ml tryptophan and in control tube. Hence 5mg/ml tryptophan concentration is optimum for plant growth. Highest wet weight (0.324g) and dry weight (0.024g) was observed at 5mg/ml L- tryptophan (Table1).

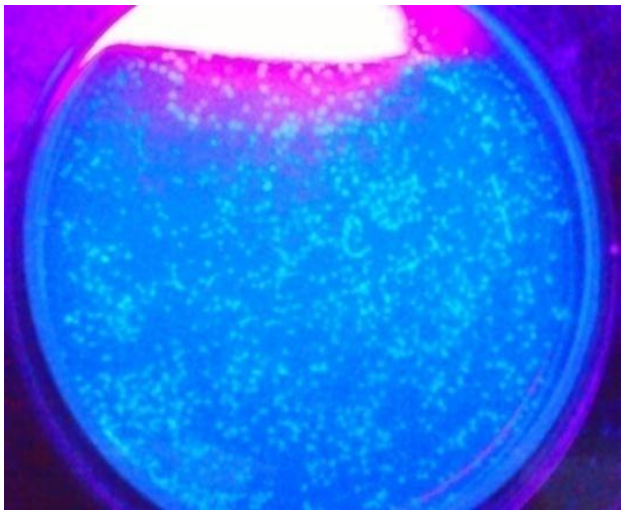

Fig 1:PGPR6 on King's B showing fluorescence under UV (365nm)

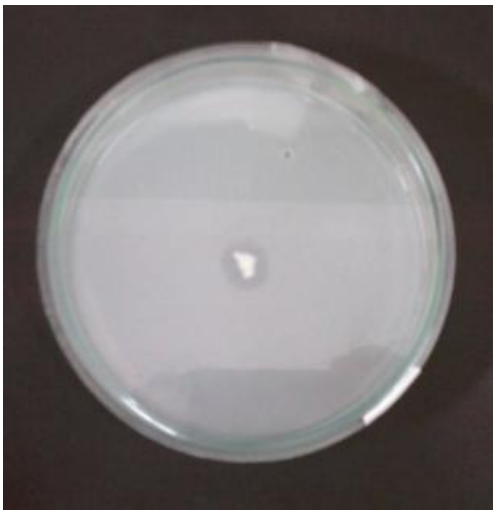

Fig 2: PGPR6 showing 3mm halo zone around colony

Fig3. Effect of different concentrations of L-tryptophan on seedling

Table 1: Effect of varying concentrations of L-tryptophan on seedlings treated with PGPR6

CONC (mg/ml)	LENGTH(cm)		WET WEIGHT(g)		DRY WEIGHT(g)	
	SHOOT	ROOT	SHOOT	ROOT	SHOOT	ROOT
CONTROL	3.5	1.5	0.036	0.014	0.002	0.001
1	5.5	3.2	0.045	0.022	0.004	0.001
3	10	3.0	0.283	0.031	0.018	0.002
5	**10.2**	**5.1**	**0.284**	**0.040**	**0.020**	**0.004**
7	9.8	4.9	0.275	0.038	0.016	0.004
9	6.5	2.8	0.150	0.027	0.007	0.002

Table2. Effect of varying concentrations of tricalcium phosphate on seedlings treated with PGPR6

CONC(%)	LENGTH (cm)		WET WEIGHT (g)		DRY WEIGHT (g)	
	SHOOT	ROOT	SHOOT	ROOT	SHOOT	ROOT
Control	10.9	3.0	0.280	0.035	0.020	0.005
0.3%	11.3	11.5	0.284	0.038	0.027	0.003
0.4%	10.6	6.3	0.283	0.092	0.026	0.005
0.5%	11.0	4.0	0.282	**0.107**	0.024	**0.006**
0.6%	11.0	3.1	0.280	0.095	0.019	0.006
0.7%	10.3	3.8	0.279	0.090	0.016	0.005

Fig4. Effect of different concentrations of tricalcium phosphate on seedlings

Fig5. Pot culture study

Effect of varying concentrations of tricalcium phosphate on the phosphate solubilising activity of PGPR6

As shown in Table2, the highest root wet weight (0.107g) and dry weight (0.006g) was observed at 0.5% tricalcium phosphate. Results reveal that all plants treated with PGPR6 showed increased shoot length, root length, dry weight, wet weight over control. Only at 0.7% concentration of tricalcium, seedling showed slight decrease in all parameters over control. Eventhough highest root length was observed at 0.3% tricalcium phosphate, the root and shoot dry weight and wet weight were considerably lower than 0.5%.

Hence 0.5% can be concluded as the optimum concentration of tricalcium phosphate required by the rhizobacteria to release highest amount of solubilised phosphate (Table2).

Pot experiment

The root length and shoot length of the mung bean plant was calculated after 10 days by taking the measurement of all the seedling growth and calculating its mean (Fig. 5). It was found that the test pot showed a shoot length of 9.92cm and root length of 6.62 cm. the germination percentage of the 'test' was found to be 100%. The plant growth promotion was assessed using Vigour index (VI).

VI = percent germination × mean total length of seedling (root length + shoot length).

Thus the vigour index of 'test' was found to be 165. In the control pot, the germination percent was found to be 85% and the mean shoot length was 3.26 cm and the mean root length was 2.87 cm. therefore by calculating the vigour index (VI) it was found to be 521.05. mung bean plant when treated with PGPR6 showed better growth characteristics as compared to 'control'. Thus it can be concluded that PGPR6 has the ability to promote plant growth. Field experimentation is required to conclusively prove the phytostimulatory potential of the isolate for its commercial exploitation as biofertiliser.

DISCUSSION

PGPR colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms. (Sivakumar *et al*., 2007). Though the exact mechanism by which PGPR stimulate plant growth is not clearly established, although several hypotheses such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilisation and promotion of the mineral nutrient uptake are usually believed to be involved (Ashrafuzzman *et al*., 2009).

IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. IAA may function as important signal molecule in regulation of plant development (Ashrafuzzman *et al*., 2009). IAA production by PGPR can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mirza *et al*., 2001). In present study, a total of 6 PGPR isolates were obtained. All PGPR strains showed positive result for IAA production. Among them, PGPR 6 was found to be good producer of IAA. Moreover isolates from rhizosphere are more efficient auxin producers than isolates from bulk soil (Sarwar *et al*., 1992). Most studies have shown that IAA biosynthesis is greatly influenced by L-TRP precursor. L-TRP is believed to be the primary precursor for formation of IAA in several microorganisms. Addition of L-TRP (an auxin precursor) to the media increased the auxin production by several fold (Farsana *et al*., 2009). IAA tryptophan monooxygenase catalyses theoxidative carboxylation of L-tryptophan to indole-3-acetamide which is hydrolyzed to indole-3-acetic acid and ammonia by indoleacetamide hydrolase via IAM pathway Hassan *et al*. (2009) stated that plants inoculated with the rhizobia together Ag+ ion and L-tryptophan gave the highest root dry weight and the uptake of N, P and K compared to non-inoculated control plants. The experiment indicated that rhizobia could be used as bioenhancer and biofertilizer for wheat production and usage of both Ag and L-tryptophan treatments together result in a significant increase on the uptake of N, P and K in comparison with using Ag+ ion and trpytophan alone and also in comparison with the blank. Patil, 2011 tested for the production of IAA in a medium with 0, 1, 2 and 5 mg/ml of tryptophan. A low amount of IAA production was recorded by *Azotobacter* strain without tryptophan addition. Production of IAA in *Azotobacter* increased with increase in tryptophan concentration from 1 to 5 mg/ml. In presence of 5 mg/ml of tryptophan, *Azotobacter* produced high levels of IAA. In the

present study, highest seedling growth was observed at 5mg/ml tryptophan concentration followed by 7mg/ml tryptophan. However a decreased growth was shown at 9mg/ml tryptophan concentration, indicating that very high concentration of tryptophan may have a negative effect on seedling growth. Very poor growth was shown by seedlings at 1mg/ml tryptophan and in control tube. The root development was highest at 5mg/ml tryptophan concentration. The amount of IAA produced by PGPR6 at 5mg/ml tryptophan was found to be 20mcg/ml.

Phosphorus is present in the form of insoluble phosphates and cannot be utilized by plants (Ashrafuzzman *et al*.,2009). Phosphate solubilisation ability of PGPR is considered to be one of the most important traits associated with plant phosphate nutrition. It is generally accepted that the mechanism of mineral phosphate solubilisation by phosphate solubilising bacterial strains is associated with release of low molecular weight organic acids, which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Chen *et al*., 2005). Among the PGPR isolates only PGPR6 showed positive for phosphate solubilising activity indicated by formation of clear halo measuring 3mm around the colony. PGPR6 solubilised 147mcg/ml of inorganic phosphate at 0.5% Ca_3PO_4. Aftab *et al*. (2005) reported that mixture of *Pseudomonas* and *Bacillus* treatments resulted in statistically significant increase in seed phosphorus content over control. Grain yield and biological yield were significantly increased by the treatments and maximum yield was recorded when bacteria was used with phosphorus alone or along with organic matter. He concluded that phosphate solubilising microorganism alone or along with other combinations produced profound effect on grain and biological yield, tillers per m^2 and seed phosphorus content. The highest root wet weight and dry weight was observed at 0.5%Ca_3PO_4. Taken together the results suggest that *Pseudomonas fluorescence* (PGPR6) is a very efficient and promising PGPR for promoting plant growth and can be used as biofertiliser. However field experimentation is required to conclusively prove the phytostimulatory potential of the isolate for its commercial exploitation as biofertiliser

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RESEARCH ARTICLE

In Vitro Callus induction and analysis of some Phytochemical parameters of *Terminalia catappa* and *Arachis hypogaea*

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Abstract

In vitro Callus induction method was developed for *Terminalia catappa* kernels and *Arachis hypogaea* nuts. They were cultured on MS medium supplemented with IAA and KiN for callus induction. The best callus induction was observed in MS medium supplemented with (IAA:KiN) (0.5:0.5) in case of *T. catappa* kernels while in case of *A. hyupogaea* nuts, it was observed with (IAA:KiN) (1.0:0.5) hormonal concentration. In addition to this, analysis of some phytochemical parameters of *T. catappa* plant parts and *A. hypogaea* nuts were evaluated in terms of Flavonoid, Anthocyanin, Chlorophyll, Proteins and Lipids. The red leaf (RL) and red epicarp (RE) showed high Antioxidants in terms of Flavonoid and Anthocyanin leading to more potent radical scavenging effect. On the other hand, Chlorophyll was found to be least in RL and RD showing that chlorophyll of the plant *T. catappa* is inversely correlated to the Antioxidants (Flavonoid and Anthocyanin). Similarly, Protein and Lipid content showed increase in red and mature parts of the plant *T. catappa.* Thus, it could be suggested that Protein and Lipids might be protected from oxidative damage due to presence of Antioxidants (Flavonoid and Anthocyanin).

Keywords: Callus, *Terminalia catappa, Arachis hypogaea,* Flavonoid, Anthocyanin, Proteins, Lipids.

INTRODUCTION

Terminalia catappa is a large tropical tree belonging to the family, Combretaceae, which is native to the tropical regions of Asia, Africa, and Australia. A deciduous or sometimes semi-evergreen tree to 15-25 m tall (Rogers and Verotta, 1996). Leaves alternate, obovate, 15-36 cm long, 8-24 cm wide, leaves turning pinkish-reddish or yellow-

brown, before falling. Fruit a somewhat compressed-ellipsoid drupe, epicarp thin, green turning yellow with a reddish blush; mesocarp fleshy, adherent to the fibrous husk of the hard-shelled stone containing the spindle-shaped seed; testa very thin, brown, enveloping the coiled cotyledons or kernel (Morton, 1985). Its fruits contain edible kernels from which high energy oil is extracted and which can also be admixed into diesel fuel (Kinoshita *et al*., 2007). It is widely planted for ornamental purposes and edible nuts (Phulwaria *et al*., 2012). The leaves contain several flavonoids, several tannins, Saponines and Phytosterols. Due to this chemical richness, the leaves (and also the bark) are used in different herbal medicines for various purposes (Hnawia *et al*., 2011). Despite its many nutritional benefits and its beneficial effects on health, processing of *Terminalia catappa* is not widespread and its consumption is limited (Biego *et al*., 2012) and means of vegetative propagation are either not known and if available have limitations. *Arachis hypogaea* L. is an annual legume belonging to family Fabaceae play a significant role in the farmers livelihoods by providing the nutritional security and fetching cash revenue (Nwokolo, 1996). Seeds have nutritional value (carbohydrates, lipids, protein, antioxidants, vitamins, essential minerals, phytochemicals and phytosterols) required for human as well as animal consumption (Atasie *et al*., 2009). Many efforts have been devoted to develop efficient *in vitro* regeneration system. It is very difficult crop to manipulate *in vitro* and only a limited success through tissue culture has been achieved (Muhammad *et al*., 2011).

Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases (Kiranmai *et al*., 2011). They are important in plant for normal growth and development (Khatiwora *et al*., 2010) and in various defense reactions to protect against abiotic stresses like UV light or biotic stresses such as predator and pathogen attacks (Rispail, 2005). Anthocyanins are a subgroup of flavonoids (Markakis, 1982). Anthocyanin pigments are important to food quality because of their contribution to color and appearance (Lee *et al*., 2005). Anthocyanin biosynthesis is often initiated due to drought, insect pests, potassium deficiency, extreme temperature, and excessive light. This behavior may allow the pigment to serve as an indicator of plant stress (Steele *et al*., 2009). Anthocyanins are becoming increasing important as antioxidant properties and health benefits, including Anti-cancer, Anti-inflammatory and Vasoprotective effects, preventing Coronary heart diseases and improving Visual acuity (Arnnok *et al*., 2012).

Micropropagation of *T. catappa* using nodal segments of 15 years old mature tree has been earlier reported by Phulwaria *et al*., 2012. For large-scale *in vitro* plant production the important attributes are the quality, cost effectiveness, maintenance of genetic fidelity, and long-term storage (Filiz *et al*., 2009). Taking the above facts into consideration, the present research work was designed to study the induction of callus and also some phytochemical parameters of *T. catappa* using its various parts and also in the seeds of *A. hypogaea.*

MATERIALS AND METHODS

Explant Collection

Both the seeds and leaves of the plant were collected from the Botanical garden of G. M. Momin Women's College. The plant was authenticated in Blatter Herbarium of ST. Xaviers College, Mumbai. The plant specimen matches with the Blatter Herbarium specimen no.16063 of H. Santapau and was identified as *T. catappa*. *A. hypogaea* pods were purchased from the local market. The seeds were used for further experimentation.

Culture Media

Basal medium used for the culture was Murashige and Skoog medium (Murashige and Skoog, 1962). It was supplemented with 3% Sucrose and 1.2%

agar. Auxin and Cytokinin were used with two different combinations [IAA: 1mg/L and Kin: 0.5mg/L], [IAA: 0.5mg/L and Kin: 0.5mg/L] and added into the medium. The medium was adjusted to pH 5.8 and autoclaved at 120°C for 20 minutes.

Inoculation of Explant

The explants were sterilized properly, the leaves were taken and their edges near to midrib were trimmed and cut into bits of 1 cm (Umamaheshwari and Lalitha, 2007) they were inoculated in Cultured Tubes under strict aseptic conditions. In the case of seeds, seed coat was separated and cotyledons were inoculated on the MS basal medium supplemented with hormones for callus induction. After inoculation, Culture Tubes were transferred to the Culture Room where the temperature adjusted was 26 ± 2°C, humidity was above 60% and light was provided with tube lights with intensity varying from 2000-4000 lux. Photoperiod given was 16 hour light and 8 hour dark.

Detection of Flavonoids

The extract was prepared according to the method given by Khatiworal *et al*., 2010. The total flavonoid content of each plant extract was estimated by aluminium chloride method. Aliquots of extracts (0.1) were taken and made up volume 3ml with methanol. Then 0.1ml aluminium chloride (10%) 0.1 ml Na-k-tartarate and 2.8ml distilled water was added sequentially. The test solution was vigorously shaken. The absorbance at 415nm was recorded after 30 min of incubation. A standard calibration plot was generated at 415nm using known concentration of quercetin (Khatiworal *et al*., 2010). The content of total flavonoids was expressed in mg of Quercetin equivalents per dry weight.

Detection of Anthocyanin

The extract was prepared according to the method given Mazandarani *et al*., 2012. The total anthocyanin content was measured by the pH-differential method (Giusti and Wrolstad, 2001). Two dilutions of berry extracts were prepared, one with potassium chloride buffer (pH 1.0), and the other with sodium acetate buffer (pH 4.5). Absorbance was measured simultaneously at 510 and 700 nm after 15 min incubation at room temperature. The content of total anthocyanins was expressed in mg of Cyanidin-3-glucoside equivalents per dry weight (Mazandarani *et al*., 2012).

Detection of Chlorophyll

It was performed according to Sadasivam and Manickam, 1996

Detection of Protein

It was performed according to Lowry *et al*., 1951

Detection of Lipids

It was performed according to Plummer, 1988

Statistical Analysis:

All assays were carried out in Triplicates and results are presented as Mean±SD.

Different parts of the plant used for various investigations are as follows:

GL(Green Leaf), RGL (Red and Green Leaf), RL(Red Leaf), RE (Red Epicarp), GE (Green Epicarp), RK (Red Kernel), GK (Green Kernel), GN (Groundnut).

RESULT AND DISCUSSION

The effect of different concentrations of IAA and KiN on callus induction of *T. catappa* and *A. hypogaea* are presented in Table.1. The best callus induction was observed when 0.5mg/l IAA and 0.5mg/l KiN was used in *T. catappa* while in *A. hypogaea* it was observed in 1.0 mg/l IAA and 0.5mg/l KiN. While in case of leaves, the nutrient medium become brown due to oxidation of phenolic compounds produced by explants.

In case of *T. catappa* both mature and immature fruit kernels were used. Results came more rapidly and efficiently in red kernel as it was more compact and mature, while layers were

separated in green kernel. In *T. catappa* (GK) with hormone concentration 1:0.5 (IAA: KiN) greening and swelling was observed after 1 week and then callus initiation was observed. Callus initiation at the edges of kernel was seen after 10-12 days. On the other hand, in RK greening and swelling was observed after 4 days and callus initiation at the edges of kernel was observed after 9-10 days. In 0.5:0.5 (IAA: KiN) hormone concentration green kernel (GK) showed no result while in red kernel (RK) greening and swelling was seen after 4-5 days and callus on the edge of kernel was seen after 1 month. Callus initiation was observed in both the kernels in the hormone concentrations 1.0: 0.5 (IAA: KiN) but callusing was little as compared to 0.5:0.5 (IAA: KiN) concentration. So it could be suggested that 0.5:0.5 is the better hormone concentration for callus induction in *T. catappa* Red fruit kernel. While in case of *A. hypogaea* in 1:0.5 concentration greening of explants was observed after 3 days and callus initiation was observed after 10 days. While in 0.5:0.5 (IAA: KiN) concentration greening of explants was seen after 4-5 days and callus initiation after 12 days. Brownish compact callus was observed in 0.5:0.5 (IAA: KiN) hormone concentration. While brown and friable callus was seen in 1:0.5 (IAA: KiN). So, it can be concluded that 1:0.5 (IAA: KiN) hormonal concentration is better for callus induction as compared to 0.5:0.5 (IAA: KiN).

Plants are conceived as sources of Antioxidants due to presence of polyphenols and flavonoids which possess wide biological properties. In the present study Flavonoid content of leaves and fruits of *T. catappa* is determined in terms of mg quercetin equivalent /g dry wt. The values were found to be high in RL i.e 23.37 mg/g as compared to RGL and GL. In case of Epicarp, it was found to be more in RE i.e 0.66 mg/g dry wt while in GE it was 0.46 mg/g dry wt. The total flavonoid content in terms of mg/g dry wt is presented in Fig 1. It was found to be absent in kernels and groundnut. Highest flavonoid content was found to be present in Red leaves. Flavonoids are most important pigments for flower coloration producing red/blue/yellow pigmentation in petals (Khatiwora *et al*., 2010). So, it can be assumed that high flavonoid content in leaves is due to the red coloration of leaves. It is reported that there is a strong correlation between the stress tolerance and antioxidant capacity in plant species (Ayşe Ş, 2012).

Table 1: Effects of IAA and Kin in MS medium on callus induction of *T. catappa* and *A. hypogaea*

Plant	Concentration of IAA mg/L	Concentration of Kin mg/L		Morphogenetic Response
T. catappa	MS+ 0	MS+ 0		No response
	MS+1.0	MS+0.5	GK	Swelling of the explants and Callus initiation from the edges after 12 days
			RK	Swelling of the explants and Callus initiation from the edges after 12 days
	MS+0.5	MS+0.5	GK	No response
			RK	Callus observed after 1 month. Callus type : Dark green whitish
A. hypogaea	MS+1.0	MS+0.5		Callus observed after 10 days. Callus type: Brown and friable
	MS+0.5	MS+0.5		Callus observed after 12 days. Callus type: Compact brownish

GK - Green fruit Kernel; RK - Red fruit Kernel

Table 2: TLC screening of Lipids in leaves and fruits of *T. catappa* and *A. hypogaea*

Adsorbent	Solvent system	Detecting reagent	Extracts	Rf values
Silica gel Precoated sheet	Petroleum ether : diethyl ether: glacial acetic acid (80:20:1)	50% v/v Sulphuric acid	GL	0.06, 0.14, 0.31, 0.63, 0.87
			RGL	0.14, 0.33, 0.62, 0.77, 0.83, 0.95
			RL	0.05, 0.15, 0.39, 0.54, 0.73, 0.86
			RE	0.06, 0.14, 0.36, 0.6, 0.72, 0.86
			GE	0.06, 0.26, 0.71, 0.97
			RK	0.1, 0.43, 0.63, 0.78
			GK	0.08, 0.52, 0.64
			GN	0.1, 0.43, 0.54, 0.76

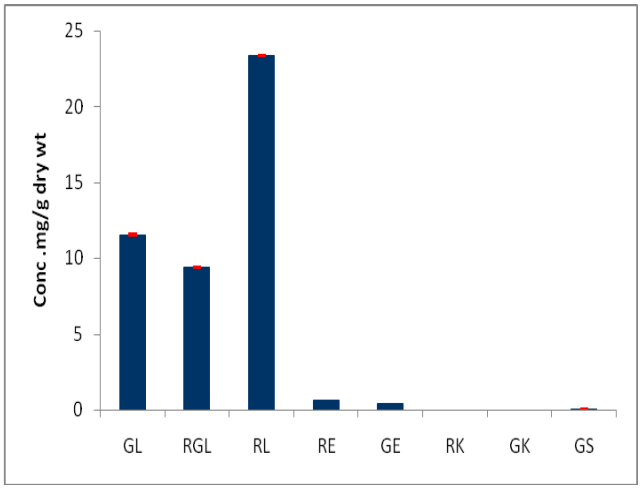

Fig 1: Determination of Flavonoid contents in various parts of *T. catappa*

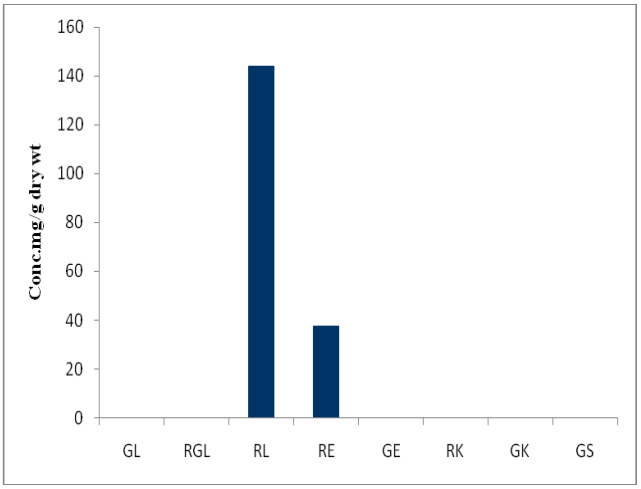

Fig. 2: Determination of Anthocyanin contents in various parts of *T. catappa*

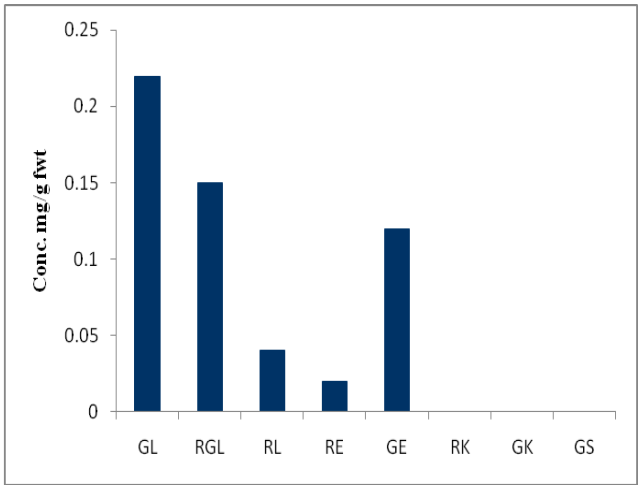

Fig. 3: Determination of Chlorophyll content in various parts of *T. catappa*

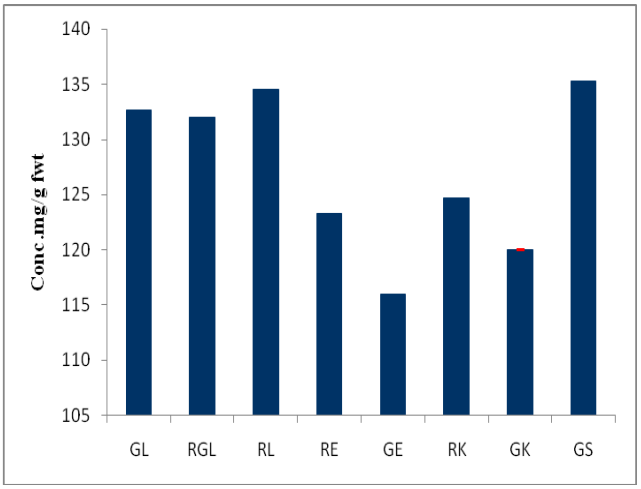

Fig. 4: Determination of protein content in various parts of *T. catappa* and *A.hypogaea*

(GL- Green leaf; RGL- Red & Green leaf; RL- Red leaf; RE – Red epicarp; GE – Green epicarp; RK – Red fruit Kernel; GK – Green fruit Kernel; GS – Groundnut seed)

Similarly Anthocyanin content was examined in various parts of *T. catappa* plant and it was found to be present only in red leaf (RL) and red epicarp (RE) and in negligible amount in kernels and groundnut. However, it has been recorded to be present in seed coat of black soyabean (Choung *et al*., 2001). It was 144 mg cyanidin-3-glucoside equivalent/g dry wt in RL while 37.5 mg/g dry wt in RE (Fig.2). In another study with rice cultivars, it was detected that under PEG induced drought stress conditions, anthocyanins, flavonoids and phenolics content increased (Basu *et al*., 2010). So, it can be concluded that plants under stress accumulates number of secondary metabolites like Anthocyanin, flavonoid etc. for its protection. Chlorophyll estimation showed reverse results to anthocyanin i.e. RL and RE contained very minute quantity of chlorophyll pigments while it was found to be highest in GL i.e. 0.22 mg/g fwt followed by RGL and GE with 0.15 mg/g fwt and 0.12 mg/g fwt. The total chlorophyll content in terms of mg chlorophyll/g tissue is presented in Fig.3. In the earlier research, it was found that under NaCl stress chlorophyll content decreased and Anthocyanin content increased (Eryilmaz, 2007). In another report, Anthocyanin accumulation increases during developmental processes (Cevahir *et al*., 2004). Present results have come in accordance with the above reports. It can be concluded that Chlorophylls and Anthocyanin are inversely proportional to each other.

Similarly, the amount of Protein and Lipid was estimated in different parts of *T. catappa* plant and it was found to be high in red parts of plant as compared to green parts. The total Protein content in terms of mg/g f wt is given in Fig. 4. It was found to be 134.66mg/g fwt in RL followed by GL and RGL with values 132.66 mg/g fwt and 132 mg/g fwt respectively. While in RE it was 123.3 mg/g fwt and in GE it was 116 mg/g fwt. In RK and GK, the values were 124.66 mg/g fwt and 120 mg/g fwt respectively. The protein content was found to be high in GN with 135.33 mg/g fwt. It can be assumed that when *T. catappa* was green and immature, protein and lipids were not synthesized but as they turn mature, its Protein and lipid synthesis increases. So, it can be assumed that protein, lipids, Anthocyanin and flavonoid are directly proportional to each other. Similarly, as Anthocyanin and flavonoids are powerful antioxidants so its increased accumulation in red leaves and fruits of *T. catappa* will protect the Proteins and lipids from any damage. On the other hand, if the plant is coming under stress, Reactive oxygen species are produced due to which cellular compartment including DNA, membrane lipids, protein may get damaged (Ayşe Ş, 2012). But due to anthocyanin and flavonoid accumulation in plants under stress condition protein and lipids are protected. Lipid estimation by TLC was done in different parts of *T. catappa* and A.hypogaea nut and to analyse number of lipid components present on the basis of their Rf values. More number of spots contributes to more number of different lipid components in different parts of *T.catappa* and *A. hypogaea* (Table.2).

Leaves and epicarp of the plant *T. catappa* showed more number of spots as compared to kernel and *A. hypogaea* nuts but they were small and thin as compared to kernel and nuts which has shown thick spots. In case of leaves, RGL and RL showed 6 spots as compared to GL which showed only 5 spots. It is possible that lipid components might have increased as leaves turn red and mature. Similarly, in case of epicarp 6 spots were visualized in RE and only 4 spots were found to be present in GE. While in fruits kernel GK showed only 3 spots and RK showed 4 spots which was much similar to GS which also showed 4 spots. The Rf values of 2 spots in RK and GS i.e. 0.1 and 0.43 were similar which might indicate the presence of same components in both the seeds. Similarly, RL showed 4 spots i.e. 0.05, 0.15, 0.73, 0.86 very much similar to the spots in RE which might also indicates the presence of same components in both RL and RE. However, lipid components in different parts of the plant *T. catappa* and *A. hypogaea* nuts are needed to be further identified.

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RESEARCH ARTICLE

State of plant biodiversity of different locations of Ambernath taluka, Thane, Maharashtra, India

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Abstract

Ambernath taluka is situated in Thane district and has a varied geographical structure. It has hills which descend from Matheran ranges. Ambernath has wide range of Plant Biodiversity & few water bodies like lakes and Ulnas River flowing through it. Apart from that Ambernath also has semi urban and well developed residential areas, as well as heavily polluted industrial areas. As the locality concern environmental factors, pollution, topography and other essential factors affect the vegetation and further the flora and fauna of the area. In the present paper we have divided the taluka into four zones on basis of their type and environmental factors and studied the plant diversity of different locations of Ambernath. The zones are as follows 1) Hilly vegetated area 2) Semi urban area 3) Urban area 4) Industrial polluted area. Taking the surrounding factors in consideration plants have been recorded and classified and the characters have been noted.

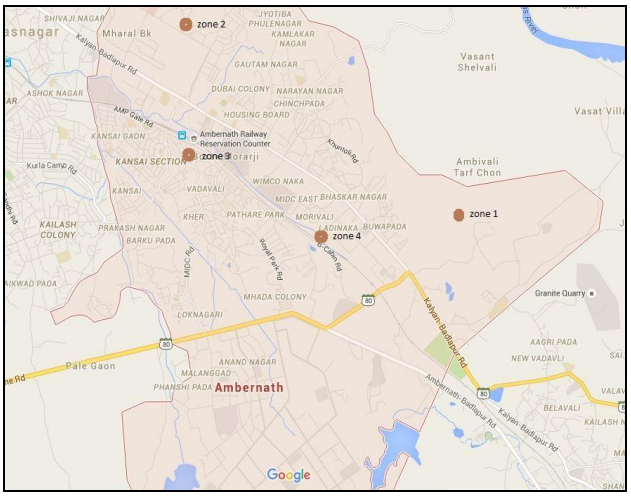
Keywords: Plant biodiversity, geographical structure, environmental factors, pollution, Ambernath, Matheran

INTRODUCTION

Ambernath falls under the plains at the foot of the slopes of the Sahayadri, and has a small part of Matheran ranges Coordinates: 19.209°N 73.186°E, having area of 38km^2 and Elevation 115ft form sea level. Ambernath descending as small hills having vegetated patches extending a few a few kilometres harbouring some wild animals. Winter is from December to February, followed by summer from March to June. The southwest monsoon season is from June to September. October and November moist deciduous type vegetation is found here. Climate in Ambernath is comparatively less humid as compared to the western part of the thane district. The temperature variation is more in the eastern part of the district comparing to the

western coastal areas (Champion and Seth, 1968) Ambernath also has well developed residential and urban areas and societies having ornamental plants and non native planted trees in. Ambernath has heavily polluted industrial areas. Mainly manufacturing matchsticks and has less vegetation and only a few type of grass that are able to survive the polluted environment.

Ambernath also has an ordnance estate which is a plain land having industries manufacturing weapons residential areas as well as dense vegetation supporting various small animals. Varied type of zones supports different type of plants and different type of fauna, which may have a crucial role on the environment and its sustainability, in the present paper we have noted the biodiversity of various types of areas and compared them.



Map of Ambernath showing different zones

MATERIALS AND METHODS

We noted the biodiversity of the different zones respectively as mentioned below

Zone 1: Hilly vegetated area - This area comprises of hills having good amount of vegetation and foot hill vegetation

Zone 2: Semi urban area – This is a residential, industrial and vegetated area where the different type of localities coexist, eg. Ordnance estate of Ambernath.

Zone 3: Urban area – This is a well developed residential area where non native plants and small weeds have been observed.eg.Ambernath (east).

Zone 4: Industrial polluted area – This is a heavily polluted industrial area where vegetation present but low plant diversity has been observed.eg. Morivali Section.

All the four zones were surveyed and plant diversity was noted. The plants observed were identified and then were compared as per different zones.

RESULTS AND DISCUSSION

Zone 1: Hilly vegetated area:

Highest amount of vegetation was found here due to lack of human intervention. This area dominated by Angiospermic plants, where flowering plants ranging about 1 to 3 meters in height and dense grass cover about 1 meter in height, trees present ranged from 4 to 6 meters in height (Melville, 1983). Some areas showed non native planted trees of *Eucalyptus* which were the tallest 10 meters and above (Takhtajan, 1969).

Zone 2: Semi urban area:

This area consists of residential industrial and vegetated areas this area this area has a varied type of vegetation. This area can be divided into two locations namely.

1. Residential and industrial: showing Bryophytes and Pteridophytes growing near walls and few inches. Residential area is dominated by native and non native fruit bearing and ornamental plants are seen at a few locations. (Singh and Mudgal, 1997)

2. Vegetated area: This area consisted of grasses similar to the hilly area and the vegetation also is similar except for some trees which were present in small patches (Takhtajan, 1980).

Zone 3: Urban area:

This area consisted of small weeds, ferns, bryophytes and pteridophytes height ranging from a few inches to one meter. Rest of the vegetation is dominated by ornamental plants.

Zone 4: Industrial polluted area

This area consist of notable amount of vegetation but the species diversity lacks, ornamental plants are seen near the industries apart from that few trees are seen at intervals and the natural vegetation is dominated by grasses and creepers. (Rao and Gupta, 1997).

Fig. 1: Image showing topography of zone 1

Fig.2: Image showing topography of zone 2

Fig,3: Image showing topography of zone 2

Fig .4 Image showing topography of zone 3

Fig.5:Image showing topography of zone 3

Table 1: Showing different types of plants

Types	Angiosperms	Gymnosperms	Pteridophytes	Bryophytes	Algae	Fungi	Lichen
No of plants	78	04	07	04	07	04	03

Fig. 6: Image showing topography of zone 4

Fig.7: Image showing topography of zone 4

Tectona grandis

Dalbergia latifolia

Lichen-Foliose

Fungi-Polyporus

Fig. 8: Different Plant Species

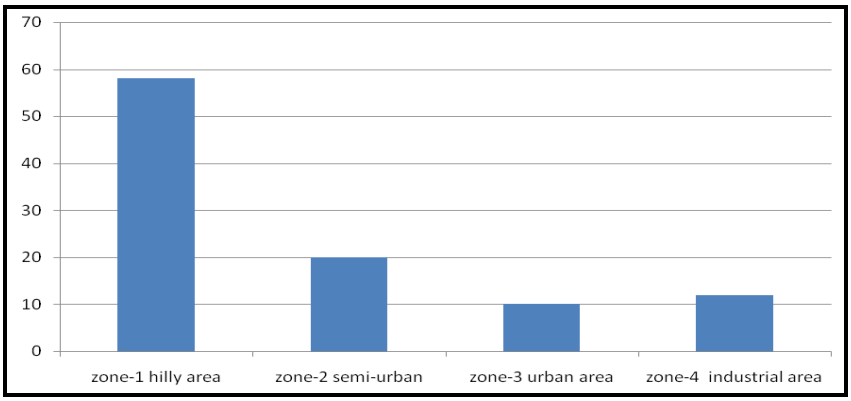


Fig.9: Diversity of respected areas in percentage

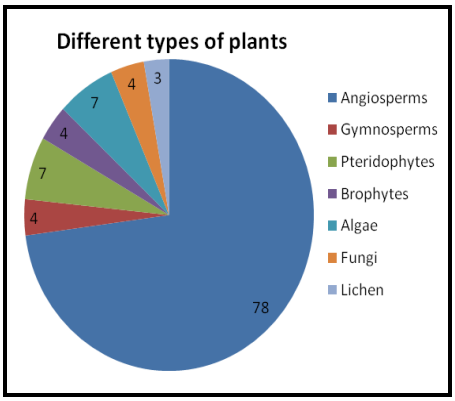


Fig. 10: Plant Percentages

CONCLUSION

After doing the survey of different zone of Ambernath the different characteristics have been observed.

Zone 1 showed highest amount of biodiversity since there is least amount of human intervention availability of vast amount of fertile land and pollution free environment

Zone 2 showed high amount of vegetation and biodiversity but lesser than zone 1 since the area had very little industrialisation and residency with a vast landscape of fertile land.

Zone 3 showed least amount of vegetation since the areas are highly populous and some amount of pollution is present here.

Zone 4 showed low amount of biodiversity but ample vegetation as compared to zone 3 since human intervention is low due to which plants that are able to survive the polluted environment are able to grow and multiply here.

Ambernath has wide range of Biodiversity showing all the types of plants which should be sustained for further research for conserving socioeconomic wealth.

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RESEARCH ARTICLE

Comparative analysis of leaf traits in two species of *Plumeria*

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Abstract

The two species belongs to genus *Plumeria* L. are *P. obtusa* and *P. pudica* has been used as an ornamental and medicinal. It is commonly known as 'White Chafa' belonging to Family Apocynaceae (Oleander family). The present paper highlights the comparison between the leaf traits (Qualitative and Quantitative) of *Plumeria obtusa* and *Plumeria pudica*. In the present study multiple morphological leaf traits such as leaf arrangement, petiole, stipule, latex, leaf surface, leaf shape, leaf margin , leaf apex, and venation were compared. The leaf size and leaf thickness were also compared. All the qualitative traits studied shows that the leaf of *P. obtusa* is glossy green, obovate, distinctive apex with entire margin whereas *P.pudica* leaf is dark green, spoon shape, acute apex with wavy margin. On comparision of quantative characters like length of the petiole and lamina of *P. obtusa* was found bigger than the *P. pudica*. The thickness of *P. obtusa* lamina was also more than *P. pudica*. It was also reported that both *Plumeria* species leaves shows spiral arrangement on the stem, presence of white milky latex , long petiole, exstipulate, and smooth leaf surface with reticulate venation. These findings are valuable in botanical identification of plant species.

Keywords: Apocynaceae, leaf traits, *Plumera* , lamina, botanical identification

INTRODUCTION

There are about 133 species of *Plumeria* L. (family Apocynaceae) reported in plumeria the plant list. It is widely cultivated in India. The two species *Plumeria* o*btusa* and *Plumeria pudica* are evergreen and is suitable to warm climate. *P. obtusa* and *P. Pudica* has been ornamentally and medicinally used. It is easily propogated by cutting as reported by Andrew *et al*. (2013) and Devprakash *et al*.

(2012). According to Tung (1999) and Scot (2009) *Plumeria* species grow as small ornamental trees in parks, residential and commercial landscapes. Medicinally leaves of *P. obtusa* shows anti mutagenic activities, antibacterial activities and used for the treatment of hyper proliferative tissue. *P. pudica* also shows anti-inflammatory and antinoceptive properties reported by Fernandes *et al*. (2015). Taxonomic classification (Chaudhary *et al*. 2014) of *Plumeria* is

Kingdom : Plantae (Plants)
Subkingdom : Tracheobionta (Vascular plants)
Superdivision : Spermatophyta (Seed plants)
Division :Magnoliophyta(Flowering plants)
Class : Magnoliopsida (Dicotyledons)
Subclass : Asteridae
Order : Gentianales
Family : Apocynaceae (Dogbane family)
Genus : ***Plumeria*** L. (Plumeria)

Very few research have been carried out in genus *Plumeria* Radha *et al*. (2008)

Plumeria obtusa (Family:Apocynaceae, Oleander family) native to West Indies including Bahamas, southern Mexico, Guatemala, and Florida. It is widely cultivated for its ornamental and fragrant flowers around the world. It secretes a milky latex sap which is poisonous and can irritate the skin. The flowers are in bouquet-like clusters of 5 white petals, a yellow center and spreading lobes.

Plumeria pudica can be grown easily and bloom heavily for a long time. Flowers are with no fragrance. *Plumeria pudica* leaves are dark green and a unique fiddle shape or spoon shape (long and thin, and widen out as a large lobes towards the tip, like the shape of a soup spoon), very short petiole, up to 13 inches long. Flowers are salverform, white, 5 overlapping petals with yellow throat up to 3 ½ inches across, and arranged on terminal cymes.

The leaves are used as a taxonomic tool for the identification of plant species. Hence on the basis of morphological features of leaves the plant species of *Plumeria pudica* and *Plumeria obtusa* are compared and identified. *P.obtusa* and *P. pudica* were also confirmed earlier on the basis of floral morphology.

MATERIALS AND METHODS

The fresh leaves of *Plumeria obtusa* and *Plumeria pudica* were collected from the different locations of kalamboli, Navi Mumbai during the month of September – October 2015. Following qualitative morphological leaf traits were studied Leaf arrangement, petiole, stipule, latex, leaf colour, leaf surface, leaf shape, leaf margin, leaf apex and venation.

Quantitative leaf traits such as leaf thickness, petiole length, lamina size (length and width) were also studied. The petiole length and the lamina size were taken as reported by Bayramzadeh *et al*. (2012). However, the width of lamina in *Plumeria pudica* was taken from the middle of a pair of broad lobes above the middle. One hundred leaves each of *P. obtusa* and *P. pudica* were studied. The Leaf thickness was measured by using Screw gauge. Petiole, lamina length and width were measured using thread and scale.

RESULTS

1. ***Plumeria obtusa***: The leaf arrangement on the stem is spirally arranged. Long petiole is present and the maximum length of the petiole measured as 7.5 cm. Stipules are absent, hence exstipulate. Presence of white milky latex observed during plucking of leaves. The leaf is glossy green in colour with an average thickness of 0.36 mm. The leaf surface of the *Plumeria obtusa* is smooth and the margin is entire with reticulate vennation. The leaf is obovate. The apex of leaf shows distinct tip and acute base. The maximum length and width of lamina measured as 35cm and 11.50cm respectively (Table 1,2 Fig.1,2,3).

2. *Plumeria pudica*: The leaf arrangement on the stem is spirally arranged. The petiole is short and maximum length of the petiole measured as 2.5cm. Stipules are absent, hence exstipulate. Presence of white milky latex observed during plucking of leaves. The leaf is dark green in colour with an average thickness of 0.20mm. The leaf surface of the *Plumeria pudica* is wavy with reticulate vennation. The leaf is spoon shape and showing acute apex and acute base. The maximum length and width of lamina measured are29.00cm and 11.00cm respectively (Table 1,2 Fig.1,2,3).

Table 1: Comparison of Morphological traits for *P. obtusa* and *P. pudica*

Morphological traits	Plumeria obtusa	Plumeria pudica
Leaf Arrangement	Spiral	Spiral
Petiole	Petiolate	Petiolate
Stipule	exstipulate	exstipulate
Latex	White milky	White milky
Leaf colour	Glossy green	Dark Green
Leaf surface	smooth	smooth
Leaf shape	obovate	spoon shape
Leaf Margin	entire	wavy
Leaf apex	Distinct tip	Acute
Venation	Reticulate venation	Reticulate venation

Table:2 Comparison of Petiole, lamina size and leaf thickness for *P. obtusa* and *P. pudica*

Quantitative Parameters	Plumeria obtusa	Plumeria pudica
Maximum lamina length (cm)	35.00	29.00
Maximum lamina width(cm)	11.50	11.00
Maximum Petiole length (cm)	7.5	2.5
Average Leaf thickness(mm)	0.36	0.20

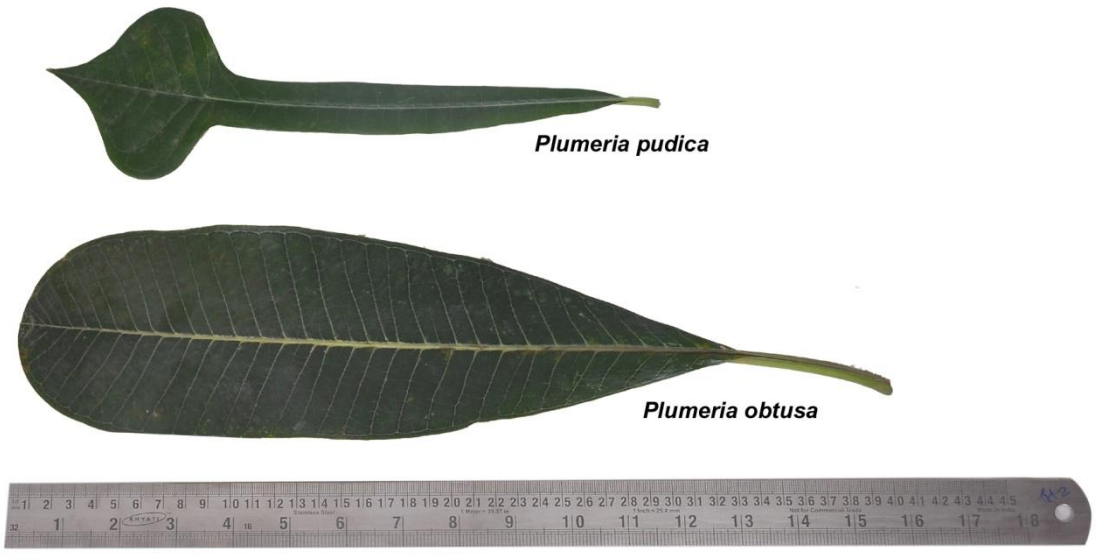

Fig 1: Leaf of *Plumeria obtusa* and *Plumeria pudica* (Dorsal view)

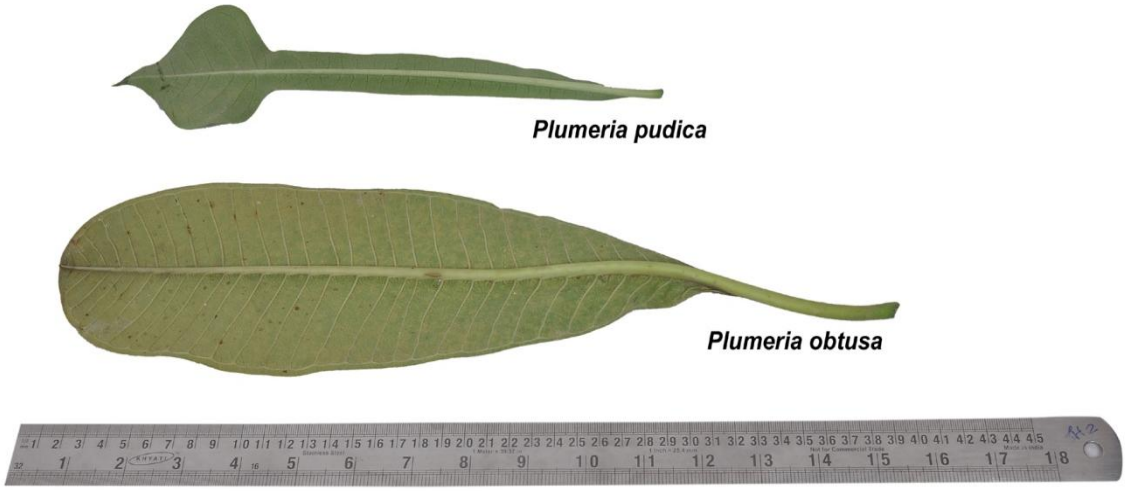

Fig 2. Leaf of *Plumeria obtusa* and *Plumeria pudica* (Ventral view)

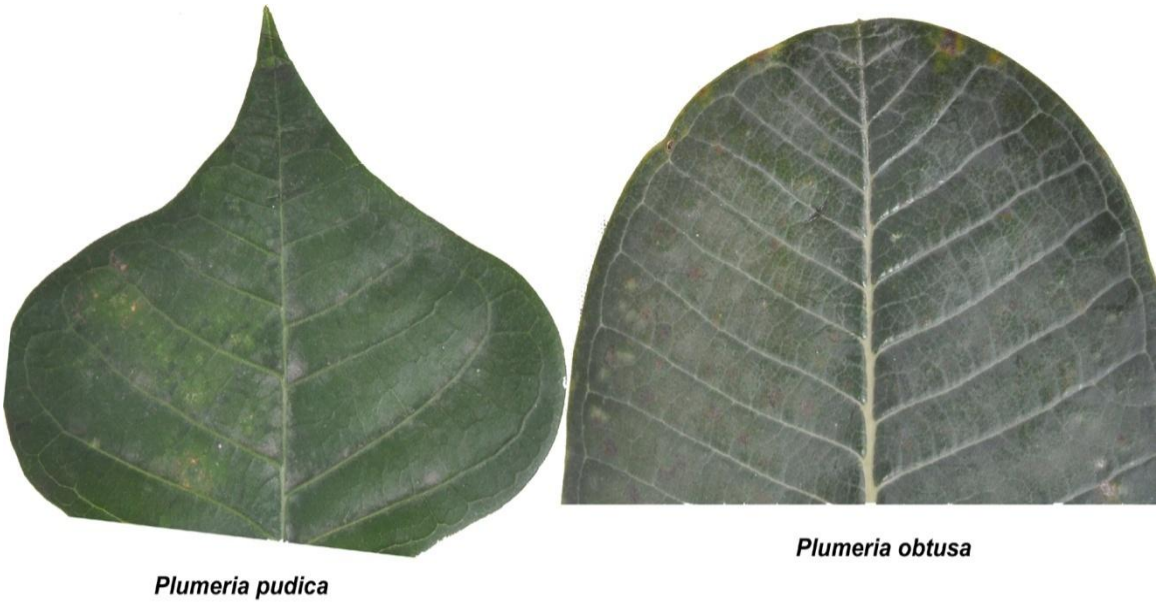

Fig 3. Leaf Apex of *Plumeria obtusa* and *plumeria pudica.*

DISCUSSION

Analysis of comparison between the leaf traits of *P. obtusa* and *P. pudica* shows that there is difference in morphological characters such as leaf colour, leaf margin, leaf shape, leaf apex and leaf thickness. In *P. obtuse,* the leaf color is glossy green, where as in *P. Pudica*, the leaf colour is dark green. Entire margin is present in *P. obtusa* whereas *P. pudica* shows wavy margin. The shape is obovate with distinct apex in *P. obtusa* and spoon shaped having acute apex in *P. pudica*. It is observed that *P. obtusa* leaf is thicker and fleshy than leaf of *P. pudica*. Certain characterstics like spiral arrangement of leaves on stem, long petiole, exstipulate and reticulate venation are

common in both the species. Presence of white milky latex observed during the plucking of leaves in both the species. (Table 1, fig 1, 2,3).

Quantitative parameters like lamina length, lamina width, leaf thickness and petiole length were also measured and found that petiole length, lamina size and thickness of leaf of *P. obtusa* is more than the *P. pudica.* (Table 2).

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RESEARCH ARTICLE

Biodiversity of *Aspergillus* spp.on Groundnut seeds

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Abstract

Groundnut seeds are generally associated with certain saprophytic or parasitic micro-organisms which perpetuate in the seed lots on the advent of favorable conditions. Several species of fungi belonging to 5 genera were recorded from seeds of cultivar of Groundnut such as *Aspergillus flavus, Aspergillus niger, Aspergillus candidus, Aspergillus ustus, Aspergillus fumigates.* Among these fungi *Aspergillus flavus, Aspergillus niger* were found to dominant and causing seed rot and seedling blight Groundnut. Here we analyzed the biodiversity of *Aspergillus* species occurring on two important Groundnut varieties viz. LGN -1 and LGN-2.

Keywards: Groundnut varieties (LGN-1, LGN-2) *Aspergillus sp.*, Blotter paper and Agar plate methods.

INTRODUCTION

Aspergillus, which is also called as "Eurotium". *Aspergillus* is widely distributed genus. They live mostly as saprophyte on almost all the dead organic material like decaying vegetables damp fruits, fatty substrata like butter, ghee, starchy materials like bread, rice, syrups, jams, jellies and wood and leather goods. Some species of *Aspergillus*, *A. flavus, Aspergillus fumigatus* and *Aspergillus niger*, cause diseases are known as Aspergilloses. Some species *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger* are responsible for causing the disease of human ear. This disease is called as Otomycosis. *Aspergillus* species are responsible for several disorders in various plant and plant products. The most common species are *A. niger* and *A. flavus*, followed by *Aspergillus candidus, Aspergillus ustus, Aspergillus fumigates, Aspergillus nidulance*. Various mycotoxins have been produced by *Aspergillus* species, the most important are the aflatoxins and ochratoxin A (Varga *et al*., 2004). Aflatoxins B_1, B_2, G_1, G_2 are the most toxic and carcinogenic

naturally occurring mycotoxins. The extensive research has been carried out on the natural occurrence, identification, character- risation, biosynthesis, and genetic regulation of aflatoxins (Bennett and Klich, 2003; Yu *et al.,* 2004).

MATERIALS AND METHODS

Collection of Seed Sample:

Seed samples of Groundnut seeds were collected from Oil research center, Latur. The following Groundnut cultivar – LGN-1 & LGN-2 were used in the study. The externally and internally seed borne fungi are identified or detected by two important method which are commonly used in laboratory and research institute. These methods are known as fallows. a. Blotter paper method. b. Agar plate method.

Blotter paper method

Doyer (1938) and De Temp (1953) were first to adopted blotter paper method in seed health management. This is the very simple, most convenient and efficient of all the incubation methods. Pair of sterile white blotter papers of 8.5 cm diameter was soaked in sterile distilled water and were placed in pre-sterilized petri-plates of 90 mm diameter. Ten seeds of test sample per petriplate were then placed at equal distance on moist blotter. 400 seeds were used in each experiment. The plates were incubated at $28^0 \pm 2^0$C under diurnal conditions. On seventh day of incubation, seeds were first examined under stereoscopic microscope for determining the various fungal growths. The identification and further confirmation of seed borne fungi was made by preparing slides of the fungi.

Agar plate method

In this method, pre sterilized petriplates were poured with 15 mL of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. Incubation and other details of the study were same as described for blotter test method.

RESULTS

In case of blotter paper methods (Table 2 and Plate I) the seeds of groundnut were associated with fungi such as *A. flavus, A. niger, A. candidus and A. ustus.* The % incidence of *A. flavus* was high (35) followed by *A. candidus* (15) and *A. niger* (10). In case of Agar plate methods (Table 3 and plate II) reveals that untreated seeds of groundnut were associated with fungi such as *A. flavus, A. niger, A. candidus and A. ustus.* and *A. fumigatus.* The % incidence of *A. niger* was high (40) followed by *A. flavus* (15) and % of low incidence were *A. ustus* and *A. fumigatus* (5). In untreated seeds of groundnut there was high % incidence of mycoflora and low % of seed germination. There was low % incidence of mycoflora but high % seed germination. *A. flavus* and *A. niger* caused discoloration of seeds and loosening of seed coat. There is also reduction in seed germination, length of radicle and length of plumule. In LGN - 2 of groundnut *A. flavus* and *A. ustus* disappear.

Table 1: Groundnut seeds associated with fungi on blotter paper (Untreated)

Sr. No.	Seed Sample	Variety	% incidence of mycoflora	% of seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi Associated
1	Groundnut	LGN-1	100	20	10.0	5.0	*A. flavus*, *A. niger* and *A. candidus*
2	Groundnut	LGN-2	60	60	12.5	6.0	*A. niger*, *A. flavus*, *A.candidus* *A. ustus*

Table 2: Effect of fungi on incidence and damage done to the groundnut seeds on blotter paper (Untreated)

Sr. No.	Fungi Associated	% incidence of mycoflora on cultivars		Nature of damage done to the seed
		LGN-1	LGN-2	
1	*A. flavus*	30	35	Discoloration of seeds and loosening of seed coat. Germination of seed is reduced.
2	*A. niger*	10	10	
3	*A. candidus*	5	15	

Table 3:Groundnut seeds associated with fungi on Agar plate (Untreated)

Sr. No.	Seed Sample	Variety	% incidence of mycoflora	% of seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi Associated
1	Groundnut	LGN-1	90	10	8.0	4.0	*A. flavus, A. ustus and A. fumigatus*
2	Groundnut	LGN-2	80	10	9.0	4.5	*A. niger and A. flavus*

Table 4: Effect of fungi on incidence and damage done to the groundnut seeds on Agar plate (Untreated)

Sr. No.	Fungi Associated	% incidence of mycoflora on cultivars		Nature of damage done to the seed
		LGN-1	LGN-2	
1	*A. flavus*	25	-	Discoloration of seeds, inhibition of seed growth, loosening of seed coat and reduction in seed germination.
2	*A. niger*	25	40	
3	*A. ustus*	5	-	
4	*A. fumigatus*	5	5	

(A) **(B)** **(C)**

Fig. 1: Showing *Aspergillus* species on Groundnut seeds.
(A) Blotter paper with infected seeds associated with *A. flavus*);
(B) Agar Plate with infected seeds associated with *A. nige*
(C) Agar Plate with infected seeds associated with *A. fumigates an A. flavus.*

DISCUSSION

In Groundnut varieties like LGN-1 and LGN-2 the fungi associated are *A.flavus*, *A. niger, A. candidus*, *A. ustus* and *A. fumigates*. The most dominant fungi in these varieties are *A. flavus* and *A. niger.* Gupta and Chohan (1976) observed seed borne fungi and seed health testing in relation to seedling disease of groundnut kernels and pod shells during storage. Mixonet et al. (1984) studied effect of *Aspergillus flavus* and Aflatoxin contamination of peanut seed. Dange and Saradava (1985) screened of selected groundnut cultivars against seed rot and collar rot caused by *Aspergillus niger.* Bansal and Sobti (1988) observed the *Aspergillus flavus* associated with groundnut seeds. Magnoli et al. (2003). studied mycoflora and ochratoxin producing strains of *Aspergillus*. Varga et al. (2004). reported molecular diversity of agriculturally important *Aspergillus* species.

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RESEARCH ARTICLE

Role of Fungi: as Ligno-cellulosic Waste Biodegrader of Agricultural waste (Paddy Straw)

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Abstract

In nature cellulose, hemicellulose and lignin are major source of plant biomass; therefore their recycling is indispensable for the carbon cycle. Each polymer degraded by a variety of microorganisms which produce a number of enzymes that work on waste material. For the present research work agricultural waste such as paddy straw were selected which was good source of lingo-cellulose content. Paddy straws were inoculated with fungal strains such as *Pleurotus sajor-caju, Trichoderma harzianum* and *Aspergillus niger and Chaetomium globosum* and allow composting process for 40 days with different combination of fungal strains. Temperature and moisture content 60-80% maintained throughout the experiment. Analysis of initial, control and compost samples showed degradation of cellulose, hemicellulose and lignin content. Cellulose decreased from 31.10% to 13.38%, hemicellulose 15% to 7.4% and lignin 14.30% to 3.35%. In the future, processes that use lingo-cellulytic enzymes or are based on microorganisms could lead to new, environment friendly technologies. This study suggests that agricultural wastes could be converted into some value added products such as compost.

Keywords: Cellulose, hemicellulose, lignin, composting, microorganisms

INTRODUCTION

In recent years, human activities have reached such a point of progress that the recycling capacity of nature has been exceeded, and the accumulation of waste has become a serious environmental and economic problem. The increasing rate of agricultural and agro-industrial waste due to the large scale of urbanization and a consequence of economic development has become a problem that

produces the huge quantities of waste in India and causes a serious environmental problem which is difficult for management.

The increasing rate at which organic wastes are generated has become a problem for disposal and/or management. Therefore, the study related on safe reuse and management of agricultural waste is important. According to Appelhof (1981), the appropriate disposal of waste should involve both maximum cost effective recovery of recyclable constituents and transformation of non-recoverable material into forms, which do not present environmental hazards.

Each year human, livestock and crop produce approximately 38 billion metric tons of organic waste worldwide. Disposal and environmental friendly management of these wastes has become a global priority. According to a conservative estimation, around 600 to 700 million tons (mt) of agricultural waste (including 272 mt of crop residues) are available in India every year, but most of it remains unutilized.

Crop residues are generated in large quantities and constitute an abundant but underutilized source of renewable biomass in agriculture. The amount of crop residues available in India is estimated to be approximately 620 million (Pandey *et al*., 2009). Half the quantity of agro-residues thus produced finds use as roofing material, animal feed, fuel and packing material, while the other half is disposed of by burning in the field. Burning agro-residues in the field is considered a cheap and easy means of disposal of excess residues. This practice appends to air pollution, increases soil erosion and decreases the efficacy of soil-applied herbicides like isoproturon (Walia *et al*., 1999).

Composting is a controlled biological decomposition process that converts organic matter to a stable, humus-like product and the process depend upon microorganisms, which utilize decomposable organic waste both as an energy and food source (Sajnanath and Sushama, 2004).

Different wastes contain high concentrations of easily degradable organic substances with high moisture content and high density. Wood and straw which has approximately 40% cellulose, 20-30% hemicelluloses and lignin (Sjostrom, 1993) is difficult to breakdown in a normal composting process and can take considerable period of time. Among the fungi, white-rot, brown rot and soft-rot fungi are more capable of degrading cellulose materials. Thus, the success of the composting process and the usefulness of compost as an organic amendment are determined by microbial enzymes like cellulose, xylanase, manganese peroxidise, lignin peroxidise and laccase which are responsible for the breakdown of several organic compounds.

Table 1: Percentage of cellulose, hemicellulose and lignin in various types of lignocellulosic wastes

Lignocellulosic waste	Cellulose (wt %)	Hemicellulose (wt %)	Lignin (wt %)
Barley straw	33.8	21.9	13.8
Corn cobs	33.7	31.9	6.1
Corn stalks	35.0	16.8	7.0
Cotton stalks	58.5	14.4	21.5
Oat straw	39.4	27.1	17.5
Rice straw	36.2	19.0	9.9
Rye straw	37.6	30.5	19.0
Soya stalks	34.5	24.8	19.8
Sugarcane bagasse	40.0	27.0	10.0
Sunflower stalks	42.1	29.7	13.4
Wheat straw	32.9	24.0	8.9

Although, the microbial community naturally present in wastes usually carries out the process satisfactorily, the inoculation of residues with lignocellulolytic microorganisms is a strategy that could potentially enhance the organic substrate, bulking agents and the amendments used in composting are mostly derived from plant material.

Composting of agricultural residues through the action of lignocellulolytic microorganisms is easier to manage and it recycles the lignocellulosic waste with high economic efficiency. Lignocellulose, the composite of the predominant polymers of vascular plant biomass, is composed of polysaccharides like cellulose and hemicelluloses and the phenolic polymer lignin. Hence, the capacity of microorganisms to assimilate organic matter depends on their ability to produce the enzymes needed for degradation of the substrate components i.e., cellulose, hemicellulose and lignin. The more complex the substrate, the more extensive and comprehensive is the enzyme system required. Through the synergistic action of microorganisms, complex organic compounds are degraded to smaller molecules, which can then be utilized by microbial cells (Golueke, 1991).

MATERIALS AND METHODS

A. Source of agricultural waste (paddy straw): After harvesting of paddy crop the plant debris remains behind that is good source of substrates used for experiment. Paddy straw was collected from local supplier, Ghatkopar (Mumbai).

B. Source of fungal bioinoculants: The 4 different fungal strains were used for the composting process. The fungal strain such as *Aspergillus niger, Trichoderma harzianum* and *Chaetomium globosum* were procured from Agharkar Research Institue (NFCCI), Pune and *Pleurotus sajor-caju* was obtained from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.

C. Culturing of fungal strains: All the four fungal strains were inoculated on PDA (Potato Dextrose Agar and Potato Dextrose Broth) plates and slants, kept in incubator at 26^0C after inoculation. After sufficient growth, all cultures were used for further composting of substrates (paddy straw).

D. Experimental set up: 15 plastic drums were prepared for experiment. 15 kg paddy straw was collected, finally chopped into small pieces and were pasteurized by dipping it overnight in 0.1% formalin. (Each drum contains 1 kg substrates).

E. Composting of paddy straw with fungal inoculants: Paddy straw substrate was inoculated with fungal strains in various combinations for composting process. Pure cultures of *Pleurotus sajor-caju, Trichoderma harzianum, Aspergillus niger, Chaetomium globosum* (1gm of mycelium of each fungal strain per 1kg substrate) were inoculated in different combinations as given experimental set up. All 15 experimental drums were divided into 5 categories such as

For Paddy Straw:
(Each category done in triplicates)
Category 1: Control (Only paddy straw) (P)
Category 2*:* Paddy straw + *Pleurotus sajor-caju* (P+*P.s*)
Category 3: Paddy straw + *Pleurotus sajor-caju +Trichoderma harzianum* (P+*P.s+T.h*)
Category 4: Paddy straw+ *Pleurotus sajor-caju + Trichoderma harzianum+Aspergillus niger* (P+*P.s+T.h+A.n*)
Category 5: Paddy straw + *Pleurotus sajor-caju + Trichoderma harzianum + Aspergillus niger + Chaetomium globossum* (P+*P.s+T.h+A.n+C.g*)

F. Factors controlling composting process: The moisture was maintained around 60-80% through the experiment. Temperature also maintained. Turning/Aeration was done manually after every 4 days.

G. Chemical analysis: Analysis of Cellulose done by Anthrone method, Hemicellulose and Lignin by Reflux method (Sadasivam and Manickam, 2005).

Fig.1: Fungal bioinoculants pure cultures from authentic Research Institutes

Fig. 2: Controlling temperature in control (Paddy straw) experimental set up

Figure 3: Composted sample of treated paddy straw after 40 days
(From left to right control P+P.s, P+P.s+T.h, P+P.s+T.h+A.n, P+P.s+T.h+A.n, P+P.s+T.h+A.n+C.g)

RESULTS

Initial paddy straw contains (Table 2) cellulose 31.10%, hemicelluloses 15% and lignin 14.30%. Paddy straw was rich source of lingo-cellulose which was beneficial for fungal growth. Paddy straw was highly lingo-cellulosic waste. The result clearly showed that (Figure 4) there was significant difference in cellulose, hemicellulose and lignin percentage after composting with fungal bioinoculants in different combinations. Cellulose percentage in control (only paddy straw) was 23.5%, and it was further decreased like 21.2% (P+*P.s*), 17.4% (P+*P.s+T.h*), 15.95% (P+*P.s+T.h+A.n*), 13.38% (P + *P.s + T.h + A.n + C.g*).

Table 2: Composition of initial paddy straw

Sr. NO.	PARAMETERS	VALUES IN PERCENTAGE
1	Cellulose	31.10
2	Hemicellulose	15.00
3	Lignin	14.30

Table 3: Composition of Paddy straw after composting with fungal bioinoculants for 40 days

PARAMETERS	Control(P)	P+ P.s	P+P.s+T.h	P+P.s+T.h+An	P+P.s+T.h+A.n+Cg
Cellulose	23.5±0.5	21.2±0.1	17.4±0.3	15.95±0.55	13.38±0.71
Hemicellulose	13.9±0.1	13.16±0.64	12.1±0	11.7±0.1	7.4±1.9
Lignin	12.7±1.20	8.65±0.45	7.85±0.15	7.3±0.3	3.35±1.15

Note: All values are mean and standard deviation of three replicates (M± SD).

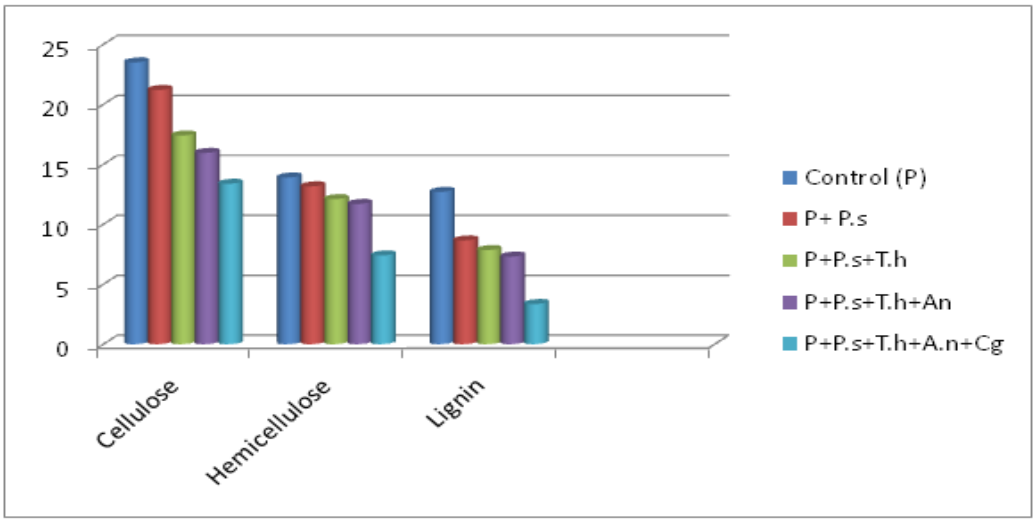

Figure 4: Comparision of cellulose, hemicellulose and lignin in different treated set up after 40 days of composting

Hemicellulose percentage in control was 13.9%, and it was further decreased like 13.16% (P+P.s), 12.1% (P+P.s+T.h), 11.7% (P+P.s+T.h+A.n) and 7.4% (P+P.s+T.h+A.n+C.g). Lignin showed 12.7% in control followed by 8.65%, 7.85%, 7.3% and 3.35% respectively as above. Dubey and Maheshwan (2005) have stated that the cellulytic fungi, such as *Aspergillus, Trichoderma, penicillium and Trichurus* accelerate composting for efficient recycling of dry crop wastes with high C:N ratio and reduce the composting period for about one month. *P. sajor-caju* has varying enzyme activities (Buswell and Chang, 1994). *Trichoderma* and *Aspergillus* degrade hemicelluloses and cellulose respectively, if these microfloras were added during pre-decomposition of the waste, the time of composting might be reduced (Singh and Sharma, 2002). Fungi are also used for remediation process, *Trichoderma virdie* proved as a good remediasing agent compared with other micro-organismas, it degrades wastes without polluting environment. Several fungi like *Trichoderma harzianum, Pleurotus ostreatus, polyporus ostriformis and Phanerochaete chrysosporium* are known to play important role in composting of lignocellulosic materials (Singh *et al*., 2012). *Chaetomium globosum*, which have ability to produce both ligninolytic and cellulolytic activity (Nagdesi and Arya, 2013). Zeng *et al*. (2010) showed the above higher activities of enzymes with *Phanerochaete chrysosporium* inoculation during agricultural waste composting. The microbial community already existed in the waste helping the composting process effectively, the inoculation of residues with ligno-cellulytic microorganisms is a another strategy that could potentially enhance the way the process takes place or the properties of the final product. Inoculation with bacteria and fungi which can

breakdown lingo-cellulolytic material has been reported to be effective in composting (Nair and Okamitsu, 2010). DanLian *et al*. (2010) tested the microbial populations and their relationship to bioconversion of lingo-cellulosic wastes during composting.

CONCLUSION

From the lingo-cellulose waste degradation point of view, the results presented above suggests that this system would be the best for agricultural and other wastes which are rich source of lingo-cellulose content. An agricultural waste has great agronomic and economic potentials as well as sustaining the environment. This study suggests that bio-stabilization of agricultural wastes with the help of fungal inoculants could be potential technology to convert noxious wastes into nutrient rich bio-fertilizer. Finally, agricultural wastes could be utilized as an efficient soil conditioner for sustainable land practices after processing by composting with fungal inoculants. This method would enable us to potentially convert the waste into value added products in a short time.

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RESEARCH ARTICLE

Study of Fungal Diversity of Regions with Anthropogenic Activity in some Green Zones of MMRDA Region

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Abstract

The kingdom fungi includes organisms that are saprophytic or parasitic. These life forms make an important tropic level of our ecosystem highly important in nutrient recycling. The current study focuses on fungal diversity in some green zones of MMRDA region with anthropogenic activity. The study also focuses on fungi associated fauna to find relation if any. The 26 species of Macrofungi and 20 species of soil fungi have found. The study shows thatthe fungal diversity in zones with limited or restricted human activity is high. An Arthropodan fauna is more associated with fungi for food. Also the diversity of fungi may depend on the plant community and soil factors, human interference such as monoculture and soil pollution may cause changes in fungal diversity of protected forest.

Key words: Fungal biodiversity associated Fauna, *Aspergillus,* Soil fungi, MMRDA region.

INTRODUCTION

There is deep association of forest community, ecological structure and fungi. Fungi provide very basic and essential services to nature including nutrient recycling, food for animals, shelter, population control of animals etc. So at one point of view to the forest community the fungi take a centre position. There are many studies conducted in relation to ecology of flowering plants and other life forms by keeping them at centre stage. But such view of study towards fungi is used by very few investigators. In the present study the fungal species found in green zones of Mumbai and surrounding regions are identified and enlisted. The data is then compared with available faunal diversity which depends on various types of fungi for food, shelter or any other association.

MATERIALS AND METHODS

The study sites were selected within Mumbai and nearby regions (MMRDA region). Four sites were selected *viz*. Sanjay Gandhi National Park (SGNP)(19°09'47"N 72°53'31"E), Mahim Nature Park (MNP) (19°03'08"N 72°51'46"E), Matheran (MTR) (18°58'50"N73°16'15"E) and Karnala forest (KRN) (18°53'37"N 73°07'01" E). The study sites were visited once a quarter (considering season of three months) from October 2012 to September 2013. The data was collected along the 2 km transect in area where human activities are quite high. The data was collected in about 5 meter distance on both side of transect. During field visits study team photographed and collected fungal specimens as per the standard methods. (Atri and Saini, 2000). Also the observations were recorded for faunal association if any. The soil samples were also collected during field visit to study microfungi in laboratory.

The identifications of macrofungi were made using available literature for fungal taxonomy such as Evans and Kibby, (2004), Christensen, (1960) and Smith (1960; Sathe and Deshpande, 1980; Thite and Patil, 1976). The identification was done by-

1. Culturing them on PDA (as per Difco Manual, 1969) by soil dilution technique. (Warcup, 1967; Pramer and Schmidt, 1966).
2. By preparing the slides stained in cotton blue in lactophenol and
3. By comparing it with the standard literature. (Nagmani *et al*, 2006).

RESULTS AND DISCUSSION

This study was focused on collecting species diversity data of fungi mainly found in regions of forest where anthropogenic activities are quite high. 25 species of Macrofungi and 9 species of soil fungi have been found in SGNP. Matheran and MNP have comparatively less species diversity in macrofungi and harbour 8 species soil fungi. The data shows that the species diversity of SNGP is higher as compared to other three regions. The diversity of soil fungi in all three regions indicates good health of forest soil around reclaimed area.

Diperanflies on *Lepiotasp.*

Beetles on *Lepiota sp.*

Fungal growth on caterpillar body

Beetles on fungal body

Flies on *Dictiophora sp*

Table I: List of region-wise fungal species.

S.N.	Fungus Species	SGNP	MNP	MTR	KRN	Can be collected in
1	*Agaricus sps.*	Jun.13, Aug.13	-	July13	Aug.13	June, July, August and if rains persist, in september
2	*Agrocybe sps.*	Jun.13, Aug.13	-	-	Aug.13	Same as above
3	*Auricularia sps.*	Aug12, Jun.13	-	-	Jun.13	Same as above + in dried forms after rainy season
4	*Cantherellus sps.*	Jun.13	-	-	-	In rainy season
5	*Coltricia sps.*	Oct.11, Dec.12, Jun.13, Aug13	Oct.12	-	Jan.13,	Grows in rainy season but persists throughout the year
6	*Coprinus sps.*	July12, Jun.13, Aug.13	Oct.12	-	-	Rainy season
7	*Coriolus sps.*	Oct.11, Aug12, Dec.12	-	July13	Jan.13	Grows in rainy season but persists throughout the year
8	*Dedalia sps.*	Oct.11, Aug13	-	-	-	Grows in rainy season but persists throughout the year
9	*Dictyophora indusiatus*	July13	-	July13	July13	Only in early rainy season
10	*Ganoderma sp.*	Aug13	-	-	Aug13	Grows in rainy season but persists throughout the year
11	*Ganoderm aaphanatum*	-	-	-	Jan.13	Grows in rainy season but persists throughout the year
12	*Geastrum sps.*	Aug.13	-	July13	-	Late in rainy season
13	*Hydropus sps.*	Sept.11,July12, Jun.13, Aug13	-	-	-	Rainy season
14	*Hypoxylon sps.*	Oct.11, Dec.12	-	-	Oct.11	Rainy season May persist even after.
15	*Lapiota sps.*	Sept.11, July12	-	-	July12	Rainy season
16	*Lycoperdon sps.*	Sept.12	-	-	-	Rainy season
17	*Marasmius sps.*	Sept.11, uly12, Aug12, Sep12, Jun.13	-	-	Jan.13	Rainy season
18	*Mycena sps.*	July12, Jun.13	-	-	-	Rainy season
19	*Phellinus sps.*	Aug 12,	-	-	Aug 12	Grows in rainy season but persists throughout the year
20	*Pleurotus sps.*	Oct.11, Jun.13, Aug13	-	-	-	Rainy season + if moisture is present in winter also
21	*Psathyrella sps.*	Jun.13	-	-	-	Rainy season
22	*Schizophyllum sps.*	Aug13	-	-	-	Rainy season
23	*Termatomyces sps.*	Oct.11	-	-	-	Grows in rainy season but persists throughout the year
24	*Thelephora sps.*	Aug 12,	-	-	Aug 12,	Grows in rainy season but persists throughout the year
25	*Volvariella sps.*	July12, Aug.13	Oct.12	-	July12,	Rainy season + if moisture is present in winter also
26	*Xylaria sps.*	Oct.11, July12, Dec.12, Jun.13	-	-	Jan.13	Can be found almost thorough out the year but grows well in rainy season

Table 2: List of soil fungi at study sites

Site	Name of the Fungus	Site	Name of the Fungus
SGNP	*1. Aspergillusniger*	**MTR**	*1. Aspergillusniger*
	2. Abisidiaglauca		*2. Aspergilluscandidus*
	3. Aspergillusflavus		*3. Aspergillusfumigatus*
	4. Aspergillusterreus		*4. Aspergillusochraceous*
	5. PenicilliumChrysogenum		*5. Chrysosporiumasperatum*
	6. Penicilliumdecumbens		*6. Fusariumsolani*
	7. Penicilliumnautatum		*7. Paecilomycesvarioti*
	8. Rhizopusstolonifer		*8. PenicilliumChrysogenum*
	9. Syncephalastrumsps	**KRN**	*1. Aspergillusniger*
	10. Trichodermaviride		*2. Aspergillusnidulans*
MNP	*1. Aspergillusniger*		*3. Chaetomiumsps.*
	2. Aspergillusterreus		*4. Cladosporiumsphaerospermum*
	3. Chrysosporiumasperatum		*5. Curvularialunata*
	4. PenicilliumChrysogenum		*6. Curvulariapallescens*
			7. PenicilliumChrysogenum

Table 3: The faunal diversity recorded on fungal body

Sr. No.	Fungus Name	Fauna recorded
1.	*Lepiota*.sp	Small Beetles Coleoptera
2.	*Auriculariasps.*	Insect like arthropods.
3.	*Dictyophorasps.*	Dipteran Flies and beetles (Diptera and Coleoptera)

The species diversity of macrofungi is indicative of forest rich in higher plant species diversity (Marcel *et al*, 1998). As perthe present investigations, the higher the plant diversity higher is the faunal diversity.

Many researchers such as Dar *et al* (2010) in case of conifer dominated forests of Kashmir, Beig *et al* (2011) From Jammu and Kashmir, Bhatt *et al* (2014) in case of Adwani forests of Garwal Himalaya, Uttarakhand, Upadhyay*et al* (2005) in case of dark spored Agarics from North Western Himalaya, have carried out biodiversity studies of macrofungi. The work by all the above authors indicates the vast biodiversity of macrofungi in different parts of India. Sangeetha *et al* (2004) had carried out studies for enhancing the yield of Paddy straw mushroom. Preservation and packaging of milky mushroom was studied by Sohliyaet al, (2010).Cultivation of jelly mushroom was tried out by Garasiya *et al*, (2007). The attempts of these authors help in conservation of some of these fungi.

SGNP and Karnala Sanctuary are protected forest areas with restricted or no settlements inside. That may have caused the lower disturbance of forest floor and lower contacts with manmade waste and chemicals. TheMatheran though part of protected forest it is highly disturbed due to anthropogenic activities. It was also found that the soil in and around Matheran region is highly mixed with horse and mule dung. That might have favoured certain species of fungi to thrive in soil. MNP has quite less disturbed forest floor but it is a manmade forest. The plant diversity and dominance pattern here is different than the natural forests. The dominant species of MNP were *Samania saman* (Fabaceae), *Pitho colobium dulci* (Fabaceae) and *Ficus benjamina (*Moraceae).

Also the land of MNP was used as dumping ground previously. Adding to this, the highly polluted Mithi rivers demarcates northern boundary of forest. This might have affected the fungal species diversity in this region. Still the team recommends detailed study of MNP forest for its soil fungi and macrofungal biodiversity to understand relations and interactions.

The Faunal diversity on fungal body majorly comprises of Arthropodan animals. The dipetran flies and coleopteran beetles are found majorly feeding on fungal body. Ants and small Arthropodans were also recorded but the purpose of their activity could not be identified.

CONCLUSION

The forests in the MMRDA region are the rich sources of fungal biodiversity. They are getting disturbed by human activities. The activities of human beings should be directed towards conservation of these species. A large number of faunal species are associated with fungi for various purposes. The protection of fungal species in turn protects the faunal diversity and balances the ecosystem.

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RESEARCH ARTICLE

Occurrence of Aeromycological Species: Dombivli-Kalyan Region

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Abstract

Airborne particles of biological origin are mainly consisting of fungal spores, pollen, bacteria, viruses, algal filaments, epidermal hairs, plant fragments etc. Fungi are the most important aero-allergens. Fungal spores' constitute a significant fraction of air borne particles. Extramural aerobiological research includes aero microbial survey at various places to identify fungi in urban environment and to study the variation in their concentration at four different sites. In the vegetable markets of metropolitan cities, rotten vegetables and fruits, gunny bags, paper bag, packing materials, straw, discarded leaves and stems forms the main substrates for the growth of airborne fungi. The culturable molds presents in the air of different sites was collected by exposing petriplates at four different sites. The maximum contributor at the aerospora was cladosporium with 8.79% contribution followed by *aspergillus* sp (6.58%) contribution tops the rank and it was followed by *Helminthosporium* (5.38%), *Aspergillus* sp (4.90%) and *Alternaria* sp with 4.47% contribution.

Key words: aerospora , fungal spores

INTRODUCTION

Aerobiology is a scientific and multidisciplinary approach focused on the biodiversity of biologically significant materials. Airborne particles of biological origin are mainly consisting of fungal spores, pollen, bacteria, viruses, algal filaments, epidermal hairs, plant fragments etc. they occur in varying concentration in the atmosphere depending upon the climatic factors, locations. Altitude and proximity to large or small water bodies. When dispersed in air they are known as aerosols.

The present study was carries out to identify culturable fungi in urban environment and to study the variation in their concentration at four different sites. In the vegetable markets of metropolitan cities, rotten vegetables and fruits, gunny bags, paper bag, packing

materials, straw, discarded leaves and stems forms the main substrates for the growth of airborne fungi. A residential are closely aggregated houses and a site in industrial area having food processing industries in the vicinity was also selected for trapping culturable fungi from the air.

MATERIALS AND METHODS

Petri plate exposure methods were used to know the status of culturable airborne fungi at different sites. The sites were sector A, sector B, sector C, and sector D. Petriplates containing potato dextrose agar as a culture medium were exposed 1 m above the ground level once in a month for 15 minutes. The petriplates after exposure were incubated at laboratory temperature for 5-7 days till sporulation. The fungal forms were identified and isolated to obtain pure cultures. The fungal colonies were counted. Identification of fungal colonies upto generic level was done on the basis of confirmed with the help of relevant literature (Gilman 1957; Barnett, 1991; Ellis 1971 and Subramanian 1971). At the time of petriplate exposure, about 30 ml of sterilized medium was poured quickly under aseptic conditions in each petriplate (size-lid O.D× height mm 100×15 and base O.D. × total height mm 94× 17) petriplate containing medium were covered with lid. Occurrence of culturable fungal colonies was correlated with metrological factors such as rainfall, relative humidity and temperature.

RESULTS AND DISCUSSSION

Volumaetric information on culturable molds present in the air of different sites was collected by exposing petriplate at four different sites in study area. Not all genera recorded on the cellotape were found growing on culture plates but only 21culturable genera were recorded. The genera such as *Aspergillus*, *Penicillium* and *Trichoderma* were precisely identified by their cultures which otherwise would have remained ignored or grouped under Aspergilli.

Highest colony count (680) was recorded with 36.17% owed by (545 colonies) with 23.13% contribution. The least colony count (220) was recorded at sector D with 11.17% contribution.

The maximum contributor of the aerospora was *Cladosporium* sp with 8.79% contribution followed by *Aspergillus* sp (6.15%) and *Helminthosporium* (5.79%) *Cladosoirium* sp with 6.58% contribution tops the rank and it was followed by *Helminthosporium* (5.38%), *Aspergillus* sp (4.90%) and *Altenaria* sp with 4.47% contribution. *Humicola* (0.21%) and *Chlamydomyces* (0.30%) contribution registered as the lowest contributor of aerospora in respective years.

Table 1: List of fungal taxa identified from the exposed petriplates at different sites

1. *Cunninghamella*
2. *Rhizopus* sp
3. *Rhizopus* sp
4. *Chaetomium*
5. *Alternaria* sp
6. *Aspergillus* sp
7. *Cercospora* sp.
8. *Chlamdomyces* sp.
9. *Cladosoirium* sp.
10. *Drechslera* sp
11. *Drechslera* sp
12. *Eoicoccum* sp.
13. *Fusarium* sp
14. *Gleotrichum* sp.
15. *Heterosporium* sp.
16. *Hetrosporium* sp.
17. *Humicola* sp
18. *Memmoniella* sp
19. *Nigrospora* sp.
20. *Paecilomuces* sp.
21. *Papularia* sp.

Maximum incidence of *Cladosporium* during monsoon was encountered, however its incidence during winter and summer season was maximum. maximum incidence of *Aspergillus* during all season was recorded . Dominance of *Curvularia* during monsoon and winter was observed whereas during summer season its higher concentration was recorded. However *Aspergillus* sp exhibited somewhat equal distribution in all seasons.

Biocomponents like fungal spores and pollen grains may initiate allergic response to susceptile individuals. Allergic people have an altered capacity to react to potential allergens, being hypersensitive to them, causing several types of eye, skin and respiratory disorders. Airborne infections and the resulting diseases threaten the lives and productivity of human beings, animals and plants. Aerobiology thus not simply means the study of microorganism in the atmosphere, but it also take into consideration the allergic properties of various boiparticles like pollen and spores. The results of the present study will be valuable in solving to cure various diseases and environmental issues.

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RESEARCH ARTICLE

Survey in relation to Ethnobotany of Ambernath Taluka of Thane district in Maharashtra state, India

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Abstract

Ambernath taluka is situated in Thane district and falls under the plains at the foot of the slopes of the Sahayadri, and has a small part of Matheran ranges descending as small hills. This area has heavy vegetation includes many Medicinal plants; many people use the plants for satisfying their needs. They have knowledge of Traditional Medicines also. The present work is carried out for the ethno medicinal plants, which were used for curing various health disorders like dysentery, diarrhea, wounds, poison bites etc. Ethnobotany, as a research field of science, has been widely used for the documentation of indigenous knowledge on the use of plants and for providing an inventory of useful plants from local flora & ethnobotanical studies have been used for the discovery of new drugs. This paper discusses ethnobotanical approach of traditional medicinal studies.

Keyword: Medicinal plants, Ethnobotany, health disorders, indigenous, Ambernath

INTRODUCTION

Since the beginning of civilization, people have used plants as medicine. Ancient people use to stay nearby river and learned first how to make agriculture and studied more forest for their food and to maintain the good health, they learned the use of plants as medicine for curing the diseases (Martin, 1995). Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996). Medicine, in several developing countries, using local traditions and beliefs, is still the mainstay of health care. Ethnobotany is the study of how people of particular culture and

region make use of indigenous plants, ethnobotany has it's roots in botany which originated from the study of plants for medicine (Schultes and Jain, 1981).

Ambernath taluka has a varied geographical structure. It has hills which descend from Matheran ranges. The hilly region of Ambernath taluka is highly rich in Plant Biodiversity, which used by local village people for fire-wood, apart from that many people are having the knowledge of medicinal plants also. The plants were mostly used for various purposes like dysentery, cold, diarrhea, snake and honey bee bite etc (Jain, 1981). The destruction of natural habitat of medicinal plants for various reasons like industrialization and urbanization, many of the medicinal plant species are threatened and striving for the existence (Jain, 1981).

MATERIALS AND METHODS

The survey of medicinal plants was done by arranging collection visits. The present work was carried out within Ambernath region, specimens were collected from field observation. Accordingly information on habit, flowering, fruiting period, medicinal value, plant part used etc. was recorded. The plants were identified with help of literature.

RESULTS AND DISCUSSION

In the present study 14 plant species were recorded. The present investigation revealed that the plant species used at various diseases occurred in different seasons for both Man as well as animals. The leaves and roots are the major parts of the plant, which are used frequently by the native people of Ambernath. Different types of preparations made from medicinally significant plants including decoction, powder, juice and paste (Almeida and Chaturvedi, 2006). Families like Malvaceae, Moraceae used dominantly. The local people of Ambernath use the medicinally important plants either as single or in combination with other plants. The following Plants have been used as a medicine by many people in Ambernath.

1. ***Botanical Name***: *Sida rhombifolia*
Family: Malvaceae
Use: Leaves used in Bronchitis, Cough, Snake-bite & inflammation. Stem used in skin disease. Root used in Diarrhea.

2. ***Botanical Name***: *Evolvulus alsinoides*
Family: Convolvulaceae
Use: The whole herb is used medicinally in the form of decoction with cumin & milk in fever & diarrhea. The leaves are made into cigarette & smoked in bronchitis & asthma.

3. ***Botanical Name***: *Cassia tora*
Family: Caesalpiniaceae
Use: Leaves used in the treatment of skin diseases like ringworm & itching. Leaves extract pain from honey bee sting bite.

4. ***Botanical Name***: *Urena lobata*
Family: Malvaceae
Use: The juice of leaves & roots is used in the treatment of diarrhea, stomach ache & dysentery. The decoction of root & leaves also taken to relieve pains due to excessive exertion. The whole plant is macerated & use externally for treatment of fracture, wounds & snake-bite.

5. ***Botanical Name***: *Mucuna pruriens*
Family: Fabaceae
Use: The plant & it's extract have been used for snake bite & treatment of Parkinson's Diseases.

6. ***Botanical Name***: *Helicteres isora*
Family: Malvaceae
Use: The roots & bark are used in the treatment of diarrhea, dysentery & diabetes. The fruit powder is used as a rich source of nutritive.

7. ***Botanical Name***: *Holarrhena antidysentrica*
Family: Apocynaceae
Use: Bark & seeds used in dysentery, diarrhea & for wounds. It is also used to treat jaundice, cold & cough.

8. ***Botanical Name***: *Azanza lampas*
Family: Malvaceae
Use: The roots & fruits are used in remedy for gonorrhea & syphilis.

9. ***Botanical Name***: *Azadirachta indica*
Family: Meliaceae
Use: Leaves boiled with water & used for bath to avoid skin infection. The young twig used as tooth brush.

10. ***Botanical Name***: *Ficus racemosa*
Family: Moraceaes
Use: Root juice is used to heal wounds & also help in bleeding.

11. ***Botanical Name***: *Ficus benghalensis*
Family: Moraceae
Use: Leaf powder mixed with coconut oil and applied on affected places to treat wound.

12. ***Botanical Name***: *Ficus religiosa*
Family: Moraceae
Use: Dried leaf powder is mixed with water taken orally to get relief from body pain.

13. ***Botanical Name***: *Phylanthus emblica*
Family: Euphorbiaceae
Use: Dried fruit powder used in diarrhea anemia, The leaf juice mixed with black pepper and drink to treat scorpion sting.

14. ***Botanical Name***: *Phyllanthus niruri*
Family: Euphorbiaceae
Use: Diuretic, laxative and cardio protective. Decoction of leaves is used for jaundice, dysentery and typhoid fever.

1. *Helicteris isora*

2. *Urena lobata*

3. *Mucuna pruriens*

4. *Azanza lampas*

5. Holarrhena antidysentrica

CONCLUSION

The impact of ethnobotany is directly related to the conservation of natural resources. Due to its interdisciplinary nature, the linkages of ethnobotany have proliferated. This valuable ethnobotanical information along with the rich bio-resources of the region needs to be conserved and taken up for sustainable utilisation. The rich bio-resources have to be translated into products and uses without which the rich resources have no value for the poor people of the region. Research must be organised on plants and diseases selected from ethnobotanic study. The survey would be helpful in photochemical studies and pharmaceutical point of view. Therefore, the need of the hour for the region is to capitalize so as to get rich indigenous wealth for the benefit of the people as well as immense scope for ethnobotanist.

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RESEARCH ARTICLE

Diversity Studies of Macrofungi and Lichens from Pangloli, Ratnagiri, Maharashtra, India

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Abstract

Pangloli is a small village in Mhasala Taluka of Raigad District, Maharashtra. The nearby cities are Mahad, Mahabaleshwar and Pen. The village maintains its traditional beauty of small individual houses with sloping red roofs amidst the orchards of coconuts, areca nuts and mangoes. It is surrounded by the hills that are green covered throughout the year. The forests in these hills are mixed deciduous. The people are mainly involved in rice cultivation but also grow some other crops. This beautiful village is changing its scenario very fast as the trekkers point and a picnic spot. A new hotel has already been established. Since the trekkers and visitors develop directly or indirectly the increase the wages of the villagers, they are also attracted by such visitors. This is resulting in the disturbances in the ecosystem of the village and the hills around. The forests in the hills possess a lot of leaf litter. Being near to the Arabian Sea, there is ample rainfall that results in maintaining abundant moisture. Both these result in luxurious growth of diverse types of fungi. This diversity is decreasing in the last few years. The present work indicates some of the species that are occurring in lesser and lesser numbers in the last few years. To carry out this work, frequent visits were made to the location especially during rainy season. The specimens were studied for their morphology and anatomy. The identification was done using standard literature.

Key words: Pangloli, Fungi, diversity, Conservation, Importance of fungi.

INTRODUCTION

The villages of Maharashtra are changing very fast due to urbanization and the development of facilities of transport. Even the small villages are catching attraction of picnic goers and trekkers.

This is leading to the economic development of the villagers. Hence these people prefer this type of growth. But there are some side effects of this. One of the most important problems is the debris of plastic waste, paper plates and broken – unbroken liquor bottles. This ruins the natural ecosystem of these spots.

Pangloli is one such small village that depicts typical structure of villages in Konkan. It is surrounded by small hills of Sahyadri ranges that are the spots of mixed deciduous forests. These forests are the sights of rare and endangered plants. Because of these plants there is a lot of leaf litter and fallen branches or dead wood in such forests. These are the materials on which typically fungi grow. Fungi being small organisms are often neglected by the conservationists. The habitats of these fungi are lost due to such human interference. The fungi play very important role in the forest ecosystem in recycling the organic and inorganic matter through the biogeochemical cycles. Many of these have medicinal properties and can be useful for the dreadful diseases such as cancer if properly studied. Many of the forest fungi are edible. They are removed from the place by the villagers without taking proper measures to conserve them. This also is responsible for the loss of species from the location. The present work is an attempt to show some of the fungal members that are declining in number from this locality.

MATERIALS AND METHODS

1. Frequent visits were madeto the location to observe and collect the specimen.
2. The standard techniques for collection and preservation were followed. (Atri and Saini, 2000).
3. Fresh specimen were used for the study of morphological characters.
4. The anatomical characters of gills, stipe, etc were studied from the specimen preserved in formalin.
5. Identification was done by using standard literature such as Christensen, (1960); Evans and Kibby, (2004) and Smith, (1960).

RESULTS

Many fungal specimen belonging to asco and basidiomycetes were collected from time to time. This helped in noting the changes in the fungal flora. Some of the most common fungi that occurred in the study area were Some species of *Agaricus, Pleurotus, Polyporus, Psythyrella, Coriolus, Marasmius, Lepiota, Xylaria, Hypoxylon, Dedalia*, puff balls, *Coprinus, Ganoderma*, etc. The leaves and other parts of the plants were also infected by the parasitic fungi. E. g. *Erysiphe* on teak leaves. But gradually the numbers of specimen of certain types were found to be declining. It is observed that a particular species is now restricted to a limited part of the forest. If this part is exposed by deforestation, that species may be lost in future. Some of the species following in this category are as follows:

***Dacryopinax spathularia*:** Synonym: *Cantharellus spathularius*. It is a member of Basidiomycetes belonging to Family Dacryomycetaceae. It is spatula shaped orange yellow coloured jelly fungus. It is 1-1.5 cm talland 2-3 cm wide. The colour changes to dark red after drying. The spores are hyaline, white, oval in shape, 8-9µ long and 2-3µ wide. Basidia are 4 spored.

***Dictyophora indusiatus* :** Synonym: *Phallus indusiatus*. It is commonly known as Bamboo fungus or bamboo pith or long net stinkhorn or veiled lady. It is a member of Basidiomycetes belonging to Family Phallaceae. The fruiting body is a conical cap of one inch width and about 1-2 cm tall on a stalk of about 5-7 cm length. The fruiting body bears an indusium in a lacy skirt like manner. The spores are associated with the cap along with a slimy substance which is known as ink. The fungus is edible- rich in proteins, carbohydrates and fibres. It occurs on dead logs

and is becoming rare and rare gradually due to habitat loss.

Tremella mesenterica **:** It is a member of Agaricales belonging to family Tremellaceae. The fruiting body is soft and gelatinous. It is an edible fungus. It develops irregularly lobed fruiting bodies. The colour varies from green, yellow or orange. It becomes dark and hard after rainy season due to drying but can retain its form after return of monsoon. Basidia are 15-18µ long. But the main form of asexual reproduction is by conidia borne on much branched conidiophores. Conidia are oval, yellow 10 X 6-7 µ in size.

Lepiota cristata : It is a member of basidiomycetes belonging to Family Agaricaceae. It is also known as stinking dapperling or stinking parasol and may be poisonous. The fruiting body is white or cream coloured, with a cap of 1-5 cm in diameter. Cap center is dark. Stipe is 2-5 cm long and 05-1 cm thick. Flesh is thin and white. Spores are white, triangular to wedge shaped, 7-8 X 3-4 µ in size.

Dictyophora indusiatus

Lepiota cristata

Tremella mesenterica

Dacryopinax spathularia

Apart from this, somecrustose, foliose and fruticose lichens were also observed at the location. Because of increased vehicular transport, the emission of sulphur compounds might have been increased hence all these specimen have disappeared but crustose lichens are still observed at the location.

Hedawooo, (2010), has reported 39 members of Basidiomycetes and four members of ascomycetes from the forests of Amravati region. Sathe and Deshpande (1980) have developed a monograph of Agaricales in Maharashtra. Upadhyay*et al* (2205) have studied dark spored Agaricales from Western Himalayas. Thite*et al* (1976) have reported 17 species of fleshy fungi from Maharashtra. Bhatt *et al* (2014) have studied the macrofungal biodiversity of Adwani forests in Garwal, Himalayas. Dar *et al* (2010) have studied the macrofungal biodiversity of Kashmir. All these studies indicate wide diversity of macrofungi that include the members of ascomycetes and basidiomycetes. This diversity is decreasing at an alarming rate due to human interference and has to be saved. The conserving measures have to be taken immediately to protect them.

CONCLUSION

There is a vast biodiversity of fungi at the selected location. The temperature, moisture content is the favourable factors but human interference is disturbing this biodiversity. It has to be conserved or else some of these species may become extinct.

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RESEARCH ARTICLE

Plant Diversity of Kalamboli area

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Abstract

Plant Diversity study is important aspect of botany. It is directly and indirectly related to the formation of environmental conditions. It also indicates the presence of dominant flora of particular area when data is analyzed. In this paper plant diversity of Kalamboli area is included. The present data includes plants of different families mostly Malvaceae, Tiliaceae, Cucurbitaceae, Asteraceae, Amaranthaceae, Solanaceae, Acanthaceae, Cruciferae, Portulaceae, Scrophulariaceae etc. The plants mentioned in the data are considered as roadside or weed plants but all of them are having some medicinal properties or useful properties and hence data collection for biodiversity should be done. It will help to make awareness among people for the conservation of such plants.

Keywords: plant diversity, conservation, dominant flora, Awareness, data collection.

INTRODUCTION

India is mega biodiversity country of the world and consists of 17,000 flowering plant species. It accounts for 8% of the global biodiversity with only 2.4% of the total land area in the world (Reddy, 2008; Hajra and Mudgal, 1997). Plants represent one of the important element of biodiversity, thus the knowledge of plant species found in the different areas of the world is a pre-requisite to conserve the ecological biodiversity. It helps us to understand the overall structure and function of an ecosystem (Sumeet *et al*., 2010). For this reason accurate and precise information of the known plant species from a given area is essential. The information is important as it allows us to prevent or avoid the potential chances of biodiversity loss and to plan future policy for the protection of our environment. According to Nair (2004) "taxonomy is an integral

component of biodiversity protection, remediation and ecodevelopment". The present study aims to highlight the plant diversity of Kalamboli area, which in turn will provide important source for use in various other fields of biology in general and botany in particular.

MATERIALS AND METHODS

To carry out work on plant diversity in Kalamboli area of Navi Mumbai in India (Figure 1), first of all, the study area was selected and divided into different regions for the sake of convenience and systematic study. A general survey of the vegetation was made and observed different plants such as herbs, shrubs and trees. Extensive field surveys were conducted in the district during different seasons through regular field visits in order to get maximum representation of the different plant species. During our field visits plant samples were collected and photographs are taken of particular species. The collected plants species were identified using of various floras of Cook (1958) and flora of Maharashtra by Almeida (1996). Additional information of plants about their habit was also recorded and incorporated in the study.

Table 1:Plant species from different families.

Sr. No	Botanical Name	Family	Common Name	Habit
1.	Heliotropium indicum	Boraginaceae	Indian heliotrop	Herb
2.	Brassica juncea	Brassicaceae	Mustard greens	Herb
3.	Portulaca oleracea	Portulaceae	Pigweed	Herb
4.	Celosia argentia	Amaranthaceae	Cocks comb	Herb
5.	Astracantha longifolia	Acanthaceae	kulikhara	Herb
6.	Muntingia calabura	Mutingiaceae	Jamaica cherry	Shrub/tree
7.	Celosia spicata	Amaranthaceae	Spiked cocks comb	Herb
8.	Sida acuta	Malvaceae	wireweed	Herb
9.	Cleome burmani	Capparaceae		Herb
10.	Cucurbita pepo	Cucurbitaceae	Pumpkin	Climber
11.	Chenopodium	Chenopodiaceae	Lamb's quarters	Herb
12.	Ipomoea purpurea	convolvulaceae	Morning glory	Climber
13.	Hibiscus esculentus	Malvaceae	Okra	Herb
14.	Ipomoea batata	Convolvulaceae	Sweet potato	Climber
15.	Malachra capitate	Malvaceae	Brazil jute	Herb
16.	Alysicarpus longifolius	Fabaceae	Moneywort	Herb
17.	Solanum melongena	Solanaceae	Eggplant	Herb
18.	Urena lobata	Malvaceae	Congo jute	Herb
19.	Impatiens minor	Balsaminaceae	Lesser balsum	Herb
20.	Momordica charantia	Cucurbitaceae	Bitter gourd	Climber
21.	Delonix regia	Caesalpinae	Gulmohar	Tree
22.	Mangifera indica	Anacardiaceae	Mango	Tree
23.	Eucalyptus	Myrtaceae	Neelgiri	Tree
24.	Ficus religiosa	Moraceae	Pipal	Tree
25.	Azadirachta indica	Miliaceae	Neem	Tree
26.	Calotropis gigentea	Asclepiadaceae	Rui	Shrub
27.	Acacia auriculiformis	Fabaceae	Earleaf acacia	Tree
28.	Moringa oleifera	Moringaceae	Shevga	Tree
29.	Peltophorum pterocarpum	Caesalpinae	Sonmohar	Tree
30.	Hibiscus rosa-sinesis	Malvaceae	Jaswand	Shrub
31.	Vinca rosea	Apocynaceae	Sadaphuli	Herb
32.	Plumeria alba	Apocynaceae	White Chapha	Tree

33.	Plumeria pudica	Apocynaceae	White Chapha	Tree
34.	Ipomoea quamoclit	Convolvulaceae	Cypress vine	Climber
35.	Lantana camara	Verbenaceae	Ghaneri	Shrub
36.	Ocimum basilicum	Lamiaceae	Tulasi	Herb
37.	Clitoria ternatea	fabaceae	Butterfly pea	Climber
38.	Ricinus communis	Euphorbiaceae	Castor	Shrub
39.	Caesalpinia pulcherrima	Fabaceae	Shankasur	Shrub
40.	Baugainvellia glabra	Nyctaginaceae	Baugainvella	Trees/shrubs

RESULT AND DISCUSSION

The study includes 40 plants from different families. The plants are categorized as herb, shrub, trees and climbers etc. Species diversity and variability of plant and animal species are the most striking feature of life, which reflects the complexity, uniqueness, and intactness of natural ecosystems (Mohammad *et al*., 2009). An appropriate biodiversity management strategy should take into account the distribution patterns of species (Perring and Lovett, 1999). Conservation of ecosystem and maintenance of biodiversity is matter of both national and international concern. The maximum numbers of plant species were identified for family Malvaceae followed by Apocynaceae, Convolvulaceae while minimum for most of the remaining family. Plants together with trees, shrubs and herbs on the earth represent one of the vital elements of biodiversity; therefore the understanding of plant species occur in the different areas of the world is a prerequirement to preserve and maintain the natural biodiversity. It helps us to appreciate the accurate information of the known plant species from a given area. The information is significant as it allows us to prevent or avoid the potential chances of biodiversity loss and to plan future policy for the protection of our environment.

CONCLUSION

Taxonomy is a great tool for identification of the different plant species. It is of fundamental importance for understanding biodiversity and ecosystem functioning, as it provides us with the data to explore and describe biodiversity through scientific analysis.

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ABSTRCATS

ABS-001

Effect of cultural conditions on *in vitro* seed germination of *Adansonia digitata* L.: A multipurpose tree species

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Abstract
Adansonia digitata (Baobab) is a fruit bearing, deciduous tree species characterized by a massive size of up to 25 meters in height and 20 meters in girth. It is not widely distributed and is restricted to a few selected pockets of India. In Madhya Pradesh, it is reported from Sagar, Bhopal and Dhar district. The plant is economically very important as it is known for its medicinal value. The present studies aim at standardizing and optimizing the effect of basal media and other conditions for establishing a protocol for *in vitro* seed germination. Mature seeds without seed coat were used as explants. MSWC seeds took 20-25 days to germinate and gave 90% germination in ½WPM media. Among the different basal media tested,M7 (½WPM liquid) gave the optimum response with 83% of the seeds germinating in 25 days. Optimum shoot length was found to be 10.2 cm in M6 medium (WPM liquid) and root length 7.06 cm in M5 medium. Cultures were initiated from the surface sterilized explants and were incubated in light in culture room adjusted at 25±2°C with 16 hours light and 8 hour dark photoperiod at 6µmol $m^{-2}s^{-1}$ light intensity. These studies will provide a means to re-establish the plant in nature and will also ensure its revival from its endangered status.

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ABS-002

In-Vitro Validation of a New Ayurvedic Tincture for Oral Health

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Abstract:
Chronic inflammation is the most common disease process to effect the periodontium and its major factor responsible for tooth loss in adults, persistence of infection of the gingival margin leads to progressive inflammation and usually to destruction of the supporting tissue. Also as herbal medicines are being used increasingly as dietary supplements to fight or prevent common disease. Considering this as fact in this study on aqueous extract of Triphala + Trikatu tincture was prepared and its antibacterial activities was tested by disc diffusion and agar plate method against pathogens such as *E.coli, S.aureus ,K.pneumoniae* , and *C. albicans* . Further effects of this tincture were screened against the isolates collected from different patients having dental plaques and were found to show antibacterial activity against *streptococcus.*

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ABS-003

Effect of Biofertilizers on physiological parameters (LAR, NAR, RGR, productivity) of *Pongamia glabra* Vent. at seeding level

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Abstract:
The two identified isolates of arbuscular mycorrhizal fungi namely *Glomus mosseae* and *Acaulospora laevis* along with *Rhizobium* isolated from the root nodules of *Pongamia glabra* Vent.were used as biofertilizers in the present investigation. The tree species selected for the investigation was *Pongamia glabra* Vent. belongs to family Leguminosae, subfamily Papilionaceae. It is a deciduous tree, reaching a height of 40-60 ft with spreading type of branches, 5-9 leaflets, ovate oblong or elliptic, acute or shortly acuminate. Flowers are pinkish white with axillary raceme. It is a tree that is well adapted to arid zones and has many traditional uses. Following physiological growth parameters were determined employing formulae: Leaf Area Ratio (LAR), Net Assimilation Rate (NAR),Relative Growth Rate (RGR), Productivity. Net productivity has been expressed in terms of grams per plant per day on the basis of dry weight. Standard deviation was calculated.

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ABS-004

Pharmacognostical evaluation of Castor leaves

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Abstract
Ricinus communis Linn. is commonly known as Castor. It is a perennial shrub. Castor belongs to the family Euphorbiaceae. The oil obtained from the seeds is well known for its medicinal properties. The therapeutic potential of the castor leaves are less known. The leaf juice is a purgative, lactagogue and emmenagogue. In order to introduce castor leaves for its medicament the current study is undertaken. The present investigation deals with the pharmacognostical studies on castor leaves. For Pharmacognostical evaluation macroscopy, microscopy, powder study and histochemical analysis of leaves were performed. Physicochemical constants such as ash and extractive values were determined. The Physicochemical analysis showed ash values 3.3 % in which the acid insoluble ash is 1 % and water soluble is 8%. The preliminary phytochemical studies revealed the presence of saponin, terpenoids, anthraquinone and cardiac glycosides.

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ABS-005

Pharmacognostical and Phytochemical Studies on Leaf, Stem and Stem Bark of *Quassia amara* (Simaroubaceae)

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Abstract
Quassia amara (amargo, bitter-ash, bitter-wood) from simaroubaceae, is a shrub or rarely a small tree. The leaves are pinnately compound with 3-5 leaflets, the leaf rachis being winged. The flowers are produced in a panicle 15–25 cm long, each flower 2.5-3.5 cm long, bright red on the outside, and white inside. The fruit is a small drupe 1-1.5 cm long. The bark contains many phytochemicals, which are 50 times bitterer than quinine. Amargo contains the phytochemical quassin, the bitterest substance found in nature. *Quassia* has a variety of uses, including treatment for diarrhea and fever. *Quassia* has antibacterial, antifungal, antifertility, antitumor, antileukemic, and insecticidal actions as well. However, efficacy in clinical trials has not been proven. The present study provides pharmagnostical and physicochemical details of the plant. These observation will enable to standardize the botanical identify of the drug in its crude form. It will also be helpful in laying down standardization and pharmacopoeial parameters, as standardization of herbal medicines is absolutely essential and is need of the hour.

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ABS-006

SMAK: DNA Extraction Technique from Seeds under Induced Abiotic Stress

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Abstract:
In the aeon of molecular genetics the study of plant species under abiotic stress facilitate development of species specific DNA markers that build on sustainability to plant under unfavourable conditions and also help the breeders to cultivate the tolerant varieties. *Luffa acutangula* is a *Cucurbitaceous* plant with several medicinal properties. The seeds of *luffa* have a hard seed coat and are rich in proteins and lipids. The prerequisite for any analytical molecular tool is DNA with high purity and yield. Abiotic stress often results in an upshot of free radicals which damage both, the genomic and proteomic contents. Also stress induced metabolites are observed to interfere and inhibit the further molecular applications of its DNA viz. PCR amplification. Thus optimization of DNA extraction protocol is the call for hour to obtain intact amplifiable DNA and development of stress related markers for *Luffa.* In present study the plant is exposed to salinity stress with different concentrations of NaCl. The DNA was extracted by two different methods, of which the optimized SMAK, yielded up to 300 ng/ µl of DNA with high purity (A_{260}/ A_{280} ratio of 1.6 -1.8). The downstream appliance of DNA extracted by also verified employing random decamer primers. The DNA isolated by SMAK method proved to be more amenable to PCR amplifications.

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ABS-007

Systematic studies on Corticolous moss flora of Trimbakeshwar, in Western Ghat, Maharashtra, India

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Abstract
Mosses which play a key role in the formation of natural biotic community and make up a significant part of species richness and plant biomass in forest. The present study deals with the occurrence of Corticolous mosses i.e. epiphytic mosses growing on bark of various trees. In this systematic study six Corticolous mosses from four families were collected during various visits of different localities at Trimbakeshwar. All the Corticolous mosses like *Brachymenium nepalense* (Hook.), *Brachymenium turgidum* Broth ex. Dix, *Diphanodonprocumbens* (C. Muell) Ren. et. Card., *Stereophyllumtavoyense* (Hook). Jaeg, *Stereophyllum ligulatum*Jaeg., *Erpodium mangiferae* C. Muell are reported from the area under investigation for the first time.

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ABS-008

Phytochemical profiling of *Cassia javanica* Linn. : An antidiabetic plant

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Abstract
Cassia javanica Linn. (Family: Leguminosae) is a popular ornamental plant grown in gardens of metropolitan cities of India. It produces beautiful pink blossoms during summer season. Apart from ornamental use, the plant yield wood for furniture. Recently leaves of *C. javanica* are proved to be antidiabetic. Being ornamental, the information regarding phytochemical aspects of leaves of this plant is meagre. Therefore in order to derive detail phytochemical composition the present work had been undertaken. The phytochemical profiling involved histochemistry, preliminary phytochemical screening, quantitative phytochemistry and thin layer chromatography (TLC) analyses of leaves. Different types of phytoconstituents were identified through histochemical and preliminary phytochemical tests. The major secondary metabolites like alkaloids, glycosides, saponins etc. were further estimated by quantitative phytochemistry. In addition to these, different fractions of secondary metabolites were separated by TLC analyses.

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ABS-009

Comparative evaluation of 'Punarnava' and its adulterant as a hepatoprotective and anti-oxidant drug

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Abstract:
The use of herbs to treat diseases is almost universal. 'Punarnava' is one such popular, ancient herbal drug known to renew and revitalize the body systems. It is used in number of disorders of liver like hepatitis, jaundice, iron deficiency anaemia, etc. *Boerhavia repens* Linn. (Nyctaginaceae) is identified as punarnava. The main pharmacological activity documented in traditional literature is its hepatoprotective activity. Besides such a medicinal importance of the drug, there appears to be confusion regarding correct identity of punarnava. Another species of the same family, *Boerhavia erecta* is also used as punarnava. However no scientific information is available about hepatoprotective property of this plant. The present work is targeted to confirm therapeutic action of these two plants by comparative pharmacological screening. The hepatoprotective activity of these two plants was evaluated in Wistar albino male rats using CCl_4 hepatotoxicity model. It includes biochemical assays of SGPT, SGOT and ALP. The liver tissue homogenate assays for GSH (Glutathione) and LPO (Lipid peroxidation) were carried out to find out antioxidant potential of the said plants.

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