DRUG DISCOVERY APPROACH ON *Tectonagrandis* Linn LEAVES AGAINST APICOMPLEXAN *Plasmodium* AND WOUND LINKS ITS ETHNOPHARMACOLOGICAL VALIDATION AND PHARMACOGNOSTICAL RELEVANCE

A dissertation submitted to

The Tamil Nadu Dr.M.G.R.MedicalUniversity
Chennai-600 032

In partial fulfilment of the requirements
for the award of the degree of

MASTER OF PHARMACY
IN
PHARMACOGNOSY

Submitted by

261220703

DEPARTMENT OF PHARMACOGNOSY

COLLEGE OF PHARMACY
MADURAI MEDICAL COLLEGE
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APRIL 2014

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CERTIFICATE

This is to certify that the dissertation entitled "**DRUG DISCOVERY APPROACH ON *Tectona grandis* Linn LEAVES AGAINST APICOMPLEXAN*Plasmodium* AND WOUND LINKS ITS ETHNOPHARMACOLOGICAL VALIDATION AND PHARMACOGNOSTICAL RELEVANCE"** submittedby**Miss.R.Jancy Gracelet (261220703)**in partial fulfilment of the requirement for the award of thedegree of **MASTER OF PHARMACY** in **PHARMACOGNOSY**by The Tamil Nadu Dr.M.G.R.Medical University is a bonafied work done by herduring the academic year 2013-2014 at the Department of Pharmacognosy, College of Pharmacy,Madurai Medical College,

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Madurai-625020

CERTIFICATE

This is to certify that the dissertation entitled **"DRUG DISCOVERY APPROACH ON *T.grandis* Linn. LEAVES AGAINST APICOMPLEXAN PLASMODIUM AND WOUND LINKS ITS ETHNOPHARMACOLOGICAL VALIDATION AND PHARMACOGNOSTICAL RELEVANCE''**submitted by**Miss.R.JancyGracelet (261220703)**in partial fulfilment of the requirement for the award of thedegree of **MASTER OF PHARMACY** in **PHARMACOGNOSY**by The Tamil Nadu Dr.M.G.R.Medical University is a bonafied work done by her under my guidance during the academic year 2013-2014 at the Department of Pharmacognosy, College of Pharmacy, Madurai Medical College,Madurai-625020.

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THE AMERICAN COLLEGE,

MADURAI-625002.

CERTIFICATE

This is to certify that the specimen brought by **Miss.R.JANCY GRACELET,** II.M.Pharmacy, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai is identified as***Tectonagrandis***Linn.belonging to the family**Verbenaceae**.

Station : Madurai. (Dr. D. STEPHEN)

Date :08-07-2013

ACKNOWLEDGEMENTS

Ifirst and foremost express my revered regard and obeisance to the **ALMIGHTY GOD**with whose blessings I was able to complete my project work.

I am grateful to express my sincere thanks to **Dr.B.SANTHA KUMAR,M.Sc(F.Sc), MD (F.M)**Dean, Madurai Medical College, Madurai, for giving an opportunity to carry outmy project work.

I heartfelt sense of gratitude to **Dr.ABDUL HASAN SATHALI, M.Pharm.,Ph.D.,**Principal I/C, College of Pharmacy, Madurai Medical College, Madurai.

I owe a great debt of gratitude and heartfelt thanks to **Dr. K. PERIYANAYAGAM, M. Pharm., Ph.D., P.G. Diploma in Clinical Pharmacy (Australia)**, Assistant Reader in Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, for his enthusiastic co-operation as my project guide and all the constant encouragement, suggestions, contribution and support rendered during the project work. As a gesture of respect I would like to extend my thanks for his patience in listening and answering to my questions.

I express my thanks and honourable regards to **Ms. R. GOWRI, M. Pharm**., **Mr. T. VENKATARATHINAKUMAR, M.Pharm., (Ph.D)**., Assistant readers inDept of Pharmacognosy College of Pharmacy, Madurai Medical College, Madurai.

I thank **Mrs.A.KRISHNAVENI,M.Pharm.,** and**Mrs.A.SETHURAMANI,M.Pharm.,** Tutors in Pharmacy,Department of Pharmacognosy, College of Pharmacy,Madurai Medical College, Madurai for their help.

I am thankful to **Mr. P. SIVAKUMAR, M.Sc., DMLT**, Lab Supervisor, **Mr.MAGUDESWARAN, DMLT**, Lab Technician and **Mrs. P. ELLAYEE**for their support during my study of this work.

I thank **Mrs. P.USHA RAVI KUMAR** and other staffmembers, Institute of pathology,Madurai Medical Collegefor their help to complete my project.

Dr. STEPHEN, M.Sc.,Ph.D.,Department of Botany, American College, Madurai for plant authentication. I place on record my gratitude to**Dr.Sasikala** Director of Siddha Central Research Institute, Arunbakkam, Chennai who helped me in the microscopic studies

I express heartfelt sense of gratitude to **Mr.P.UDAYA KUMAR,** District Malaria Officer,Ramnad supply Plasmodium falciparun to carryout my bioassay.

I also thank my ever loving classmates**Ms.P.ANITHA**,**Mr.S. JEGADEESHMs.D. SUGANYA, Ms.KALAIYARASI, Mrs.S.R. NANDHINI BOSE, Mrs. S. NATHIYA PRAKASH, Ms. K.VIJAYALAKSHMI**, **Ms.E.AJILA, Ms.R.ELAVARASI, Ms.S.KARPAGAM, Mr.P.KANNIAPPAN** and my juniors**Mr.SEVA KUMAR, Mr.BALA SUBRAMANIYAM, Mrs.PREMALATHA, Mrs.VIJAYALAKSHMI, Ms.GOKILA** for their constant motivation and help.

My heartfelt thanks to my lovable friend Miss **P.BALA**, Department of Pharmacognosy, Madurai Medical College, Madurai for the help and encouragement, endless patience to complete my work successfully.

I thank to **Mr.PRABHAKAR RAO,M.pharm.,**Pavan college of pharmacy,kolar for his motivation to complete my studies.

I also thanks to my friends **SUJI, SARASU, JEMI, DEEPA, SHAMILI, SHYNI**for their encouragement and co-operation to my studies.

Above all, I am foreverindebted to myparentsfor their understanding, endless patience,help and encouragement which made me to complete this work in a successful manner.

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CHAPTER – I

INTRODUCTION

The role of Medicinal plants in the Human history:

Human relied on plants for basic needs such as food,clothing and shelter, all products of plant matrices such as leaves, woods, fibres etc and storage parts like fruits, tubers for many centuries. Plants was also used for additional purposes like arrow and dart poisons for hunting, poisons for murder, hallucinogens used for ritualistic purposes, stimulants for endurance hunger suppression as well as inebriants and medicines. The plant constituents used for these latter purposes are largely secondary metabolites (like alkaloids,flavonoids,glycosides etc.) which are derived biosynthetically from plant primary metabolites (e.g carbohydrates, aminoacids and lipids) and are not directly involved in the growth, development or reproduction of plants.

Arrow and dart poisons have been used in certain parts of the world with the principal ingredients derived from the genera *Aconitum, Antiaris, Strophanthus, Strychnos* etc. In some cultures some plants well documented for murder were henbane, mandrake, deadly nightshade, calabar bean etc. Certain plants formerly used for arrow poisons have also been used as medicines at lower dosages due to their desirable pharmacological actions.[Bisset NG., 1991]

The hallucinogens used in the past was usually associated with magic and ritual eg*Cannabis sativa, Erythroxylum coca, Papaversomniferum* etc. Tea, coffee, cocoa, cola were used in the production of stimulant beverages and inebriants or intoxicants (Wine, beer, kava) in many cultures since ancient times.[SneaderW .,1996]

Plants were the basis of sophisticated traditional medicine (TM) practices and used for thousands of years in India, China and many other countries. Some of the earliest records of the usage of plants as medicines are found in the Atharvaveda (which is the basis of Ayurveda) in India, the clay tablets in Mesopotamia and the Eber Papyrus in Egypt, De MateriaMedica of Dioscorides, Pen Ts'aoChing Classic of MateriaMedica.[Nerukar PV *et al.*, 2004,Sneader W 2005].

Before the realization that pharmacologically active phytoconstituents present in the plants are responsible for their effect, the "Doctrine of Signatures" was used to identify plants for treating diseases, for e.g. golden rod with yellow hue was used to treat jaundice, red coloured herbs for blood diseases, liverworts for liver diseases, pile worts for hemorrhoids, toothworts for tooth ache. [Sneader.,2005]

Morphine, atropine, caffeine, cocaine, ephedrine, pilocarpine, physostigmine, quinine, salicin, theobromine, theophylline, tubocurarine were isolated in 19th century.[Sneader W1996, Samuelsson G 2004].

The correlation between the ethnomedical usage of herbs and modern medicines discovered from them has been studied . In this analysis 88 single chemical entities isolated from 72 medicinal plants was introduced in to modern therapy many of which have the same or similar therapeutic purpose as their original ethnomedical use. [Fabricant., DS and Farnsworth NR., 2001].Some of these plant derived constituents such as atropine, codeine, morphine, pilocarpine etc. are still being used widely as single agent or combination formulations in prescription drugs [Snedear W.,1996]. Now a days , plants are still important sources of medicines, this era especially in developing countries that still use plant derived TM for their healthcare.

Plant based compounds and their role in drug development:

In spite of the recent resurgence of interest in drug discovery by molecular modeling, combinatorial chemistry and other synthetic chemistry methods, natural product derived compounds are still proving to be an invaluable source of medicine for humans. Other than the direct usage of plant based metabolites in their original forms as drugs, these compounds can also be used as drug precursors, templates for synthetic modification and pharmacophores all of which will be discussed briefly here.

Secondary metabolites from plants as drug precursors:

Natural products derived from plants can be used as small molecule drug precursors which can be converted into the compound of interest by chemical modifications or fermentation methods. The semisynthetic approach is a valuable to resolve the shortage of supply due to the low yield of compounds from plants and or the high cost of total synthesis. The following are some secondary metabolites from plants which are useful drug precursors, though they are not necessarily pharmacologically active form naturally. To meet the market demand of the anti-tumour drug paclitaxel cropping of the bark of slow growing Pacific yew tree ,*Taxusbrevifolia* Nutt. and the synthesis are not feasible method to provide sufficient amounts. But 10-deacetylbaccain III can be isolated comparatively larger amounts from the needles of the other related yew species, such as *Taxusbaccata L.*, a renewable resource, can be converted chemically in several steps into paclitaxel.[Denis JN,Greene AE1988, Holton RA*et al.,* 1995].

Diosgenin, a steroidal sapogenin obtained from the tubers of various *Dioscorea*species that grow in Mexico and Central America is chemically converted into

progesterone which is the key intermediate for the production of cortisone an important anti-inflammatory.

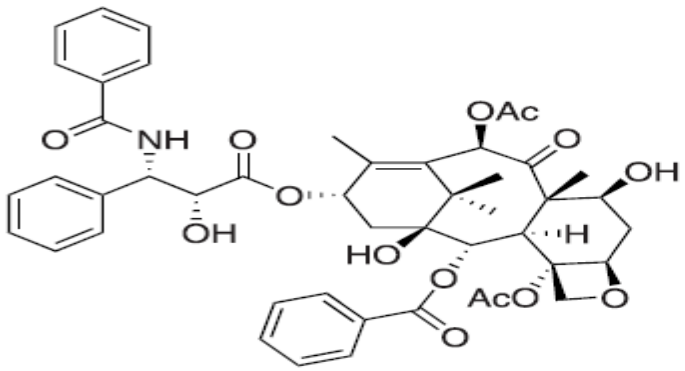
[Mancera O*et al*., 1952, 1953].

Tamiflu (Oseltamivir phosphate) an orally active neuraminidase inhibitor developed for the prophylaxis and treatment of influenza viruses A& B is synthesized from (-) shikimic acid which is an important biochemical intermediate in plants and microorganisms. Though initially it was isolated from star anise, shikimi tree (*Illiciumverum*), later on it was obtained from the fermentation of genetically engineered *E.coli* strains.Currently, Roche, the drug manufacturer still relies on both extraction and fermentation methods to obtain ton quantities of shikimic acid. [AbrechtS *et al*., 2004,Yarnell A., 2005].

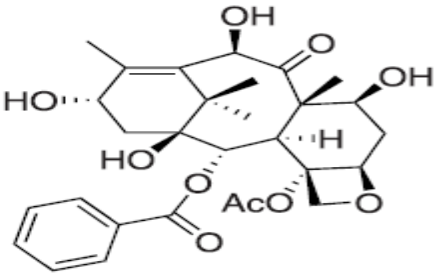
Secondary metabolites from plants as drug precursors:

Prototype defined as " the first compound discovered in a series of chemically related therapeutic agents" [Sneader W., 1998]. Podophyllotoxin, camptothecin and guanidine are drug prototypes with analogs having the same pharmacological action as the parent compound while atropine is a drug prototype that has furnished many analogs that have additional pharmacological properties. Guanidine is a natural product with good hypoglycemic activity isolated from *Galegaofficinalis* but is too toxic for clinical use. Though many derivatives of guanidine have been synthesized metformin (dimethylbiguanide) was later found to be clinically suitable for treatment of type II diabetes.[Krentz AJ and Bailey CJ., 2005].

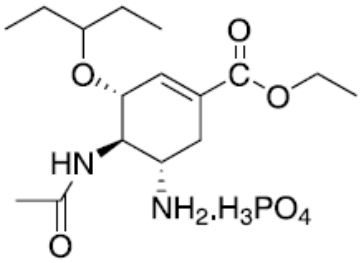
Taxol



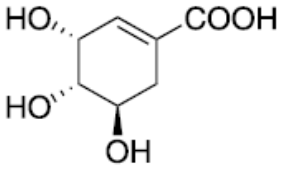
10-deacetyl baccatin



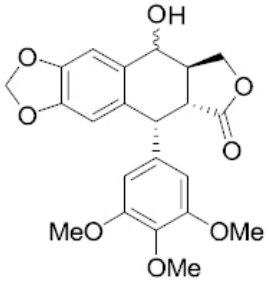
Tamiflu



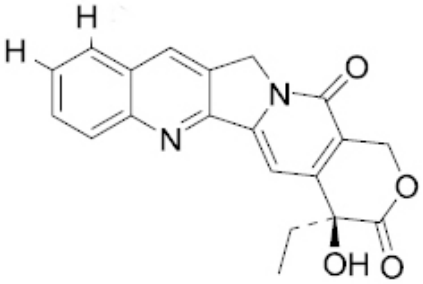
Shikimic acid



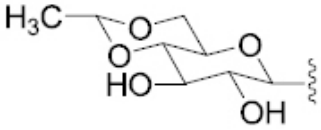
Podophyllotoxin



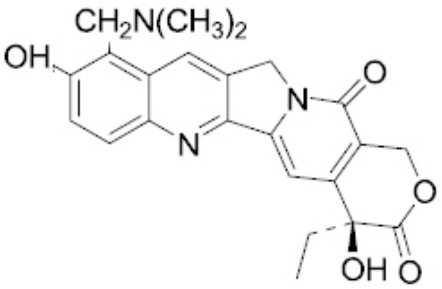
Camptothecin



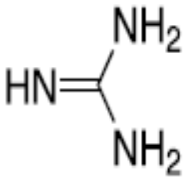
Vepesid



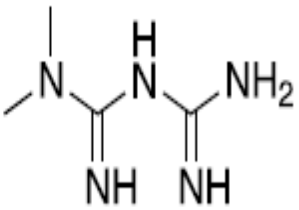
Hycamtin



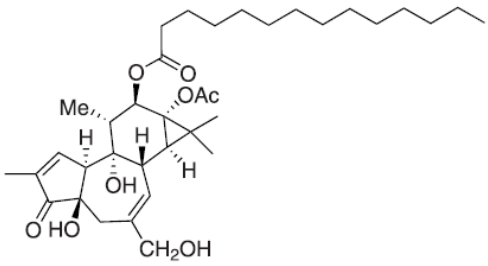
Guanidine



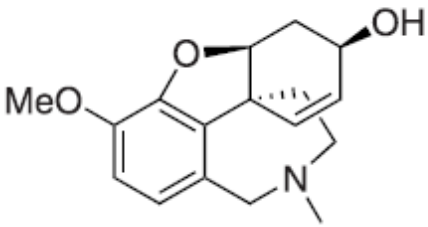
Dimethyl biguanide



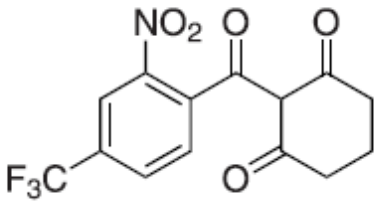
12-*O*-tetradecanoylphorbol-13 acetate



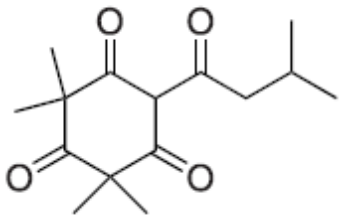
Galantamine



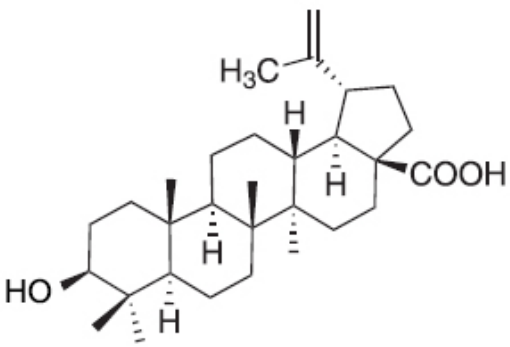
Nitisinone



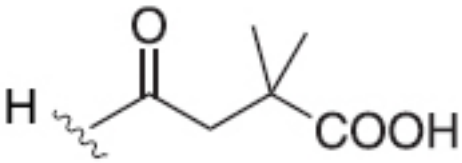
Leptospermone



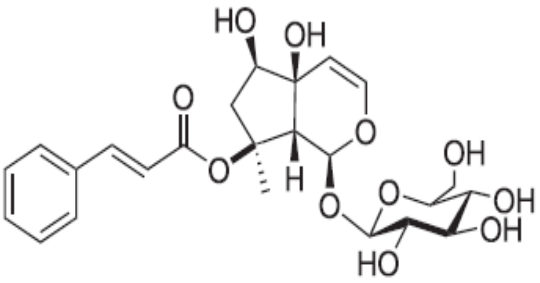
Betulinic acid



Bevirimat

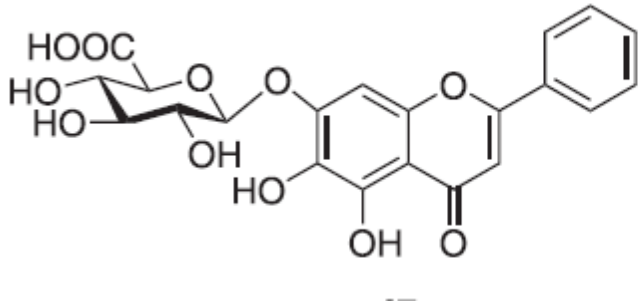


Harpagoside

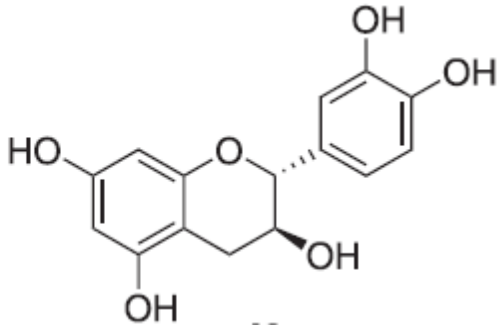


Baicalin

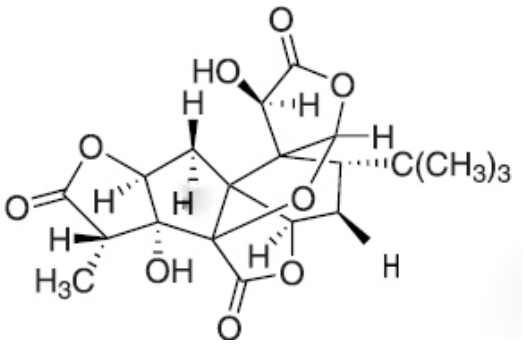
Cathechin



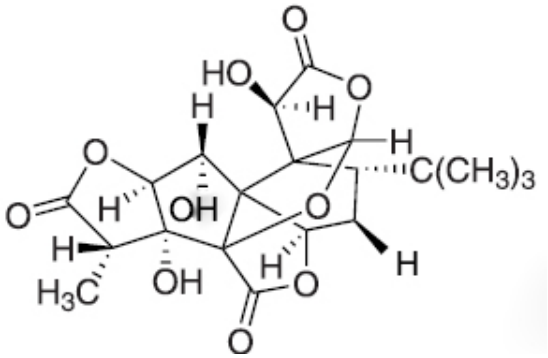
Ginkgolides A



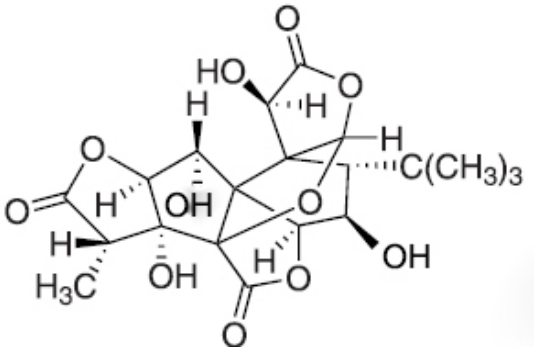
Ginkgolides B

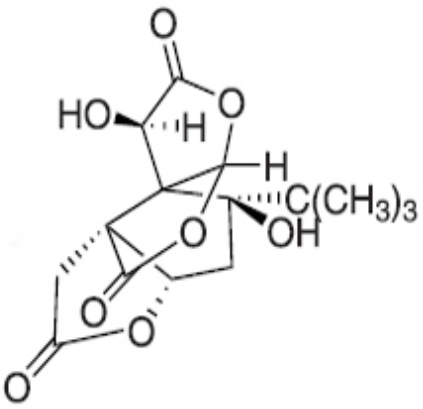


Ginkgolides C



Bilobalide





Secondary metabolites from plants as pharmacological probes:

Secondary metabolites of plant origin like phorbol esters and genistein can be uses as “pharmacological probes” which help researchers to understand the mechanism of action of intracellular signal transductions and biological mechanisms related to human diseases which can aid the design of better drugs. An isoflavone found naturally in soyabean,

Genistein, is an inhibitor of various protein tyrosine kinase (PTK) which are essential enzymes involved in intracellular signal transduction. Genistein has been used to probe the interaction between PTK and cyclic nucleotide gated channels which are important in mammalian olfactory and visual systems. Phorbol, a tetracyclic diterpenoid plant secondary metabolite isolated as hydrolysis product of croton oil from the seeds of *Croton tiglium* has been used in biomedical research as carcinogen promotions [Molokanova E and Kramer RH., 2000].

Drug discovery from plants- recent developments:

Plant derived natural products still contribute to the overall total number of new chemical entities(NCE) that continue to be launched to the market.

Plant derived drugs launched from 2001:

In the past 6yrs five new drugs derived from plants namely apomorphinehydrochloride(for Parkinson's disease), galantaminehydrobromide(for early onset of Alzheimer's disease), nitisinone (for hereditary tyrosinemia type1), tiotropium bromide(bronchospasm with COPD and varenicline(aid to smoking cessation) have been approved by the US FDA.

Plant derived compounds involved currently in clinical trials:

Many medicinal plant-derived compounds are currently undergoing clinical trial for the potential treatment of various diseases in which majority are in the oncological area including new analogs of known anticancer drugs based on the camptothecin, taxane-podophyllotoxin or vinblastin type skeletons. Compounds with carbon skeletons different from the existing plant derived drugs used in cancer are betulinc acid, ceflatonine,

combretastatin A4 phosphate, ingenol-3 angelate, phenoxodiol and protopanaxadiol.Bevrimat and celgosivir are currently undergoing clinical trials for the treatment of HIV and hepatitis C viral (HCV) infections respectively. Capsaicin is in clinical trial for the treatment of severe postoperative pain and huperzine is developed for Alzheimer's disease. Betulinic acid is a lupine type triterpene that is mostly distributed in the plant kingdom and this constituent along with various derivatives shown to have anti-inflammatory, anticancer, antimalarial, anti-HIV, anthelminthic, and antioxidant properties. (Cichewicz RH and Kouzi SA., 2004). In 1995, University of Illinois at Chicago research group reported that betulinc acid inhibited human melanoma both in vitro and *in-vivo* systems and induced apoptosis in Mel-2 human melanoma cells. It was further developed under the Rapid Access to Intervention Development program of the United States National Cancer Institute and currently undergoing I/II clinical trials for the treatment of dysplatic melanocytic nevi, a preliminary symptom that may lead to melanomas of the skin. Bevirimat a semisynthetic compound derived from betulinc acid is being developed by Panacos Pharmaceuticals, Watertown, MA, USA as a new class of antiretroviral drug. Bevirimat blocks HIV-1 maturation by distrupting a late step in the *Gag* processing pathway, causing the virons released to be noninfectious, thus terminating the viral replication. It is currently undergoing Phase II clinical trials and Phase III trials are expected to start soon. (www .clinicaltrials .gov/ct /show/ NCT00346502, www.panacos.com)

Plant extracts currently involved in clinical trials:

New forms of registered phytomedicines are not single chemical entities. These complex drugs are subjected to quality control via extract standardization procedures involving either or both constituents with known biological activity or inactive marker

compounds present in high concentration. *Harpagophytumprocumbens*, Devil's claw extract from Africa is currently undergoing phase II clinical trials in the USA for the treatment of hip and knee osteoarthritis. Flavocoxid (Limbrel) a proprietary blend of natural flavonoids from *Scutellariabaicalensis* and *Acacia catechu* is being marketed in the USA by Primus Pharmaceutical under prescription as a medical food therapy for osteoarthritis Ginkgo extracts from the dried leaves of *Ginkgo biloba* used for the treatment of early stage of Alzhemer's disease, vascular dementia, peripheral claudication, tinniturs of vascular origin. Mistletoe (*Viscum album*) extract used in the treatment of cancer in European countries. Sativex developed by GW pharmaceuticals is Cannabis sativa extract for the treatment of neuropathic pain in patients with multiple sclerosis.(www.Clinicaltrials.gov/ct/gui/show/NCT00391079).

Hence it is clear that natural products are an integral part of human health care system nowadays because there is now popular concern over toxicity and side effects modern drugs. The untapped wealth of the plant kingdom has become a target for the research by multinational drug companies and research institutes for new drugs and lead compounds.

There is recent increased participation of pharmaceutical companies in the antimalarial drug discovery and development process offers hope for the development of new, affordable drugs. Indeed, an unprecedented number of malaria discovery and development projects are now executed, involving many organizations including the Medicines for Malaria Venture (MMV) as malaria remains one of the most important diseases of the developing countries killing 1-3 million people and causing disease in 300-500 million people annually.

Recent malaria discovery and development projects and associated organizations[Fidock, DA et al., 2004].

Discovery projects	**Development projects**
Improved quinoline GSK, Liverpool	Rectal artesunates
Farnesyltransferase inhibitors BMS,Wasington	Chlorproguanil, dapsone,artesunate
Manzamine derivatives	Pyronaridineartesuanate
Cysteine protease inhibitors GSK	Amodiaquineartesunate
Fatty acid biosynthesis inhibitionMMV,Texas A&M U., AECOM Jacobus	
	Mefloquineartesunate
Chalcones (National U.Singapore, LicaPharmaceuticals)Short chainchloroquine	
third generationantifolates.	

The failure to eradicate malaria is due to a number of factors including resistance of the causative vector female *Anopheles* mosquitoes to insecticides such as DDT and avoidance of insecticide use because of toxicological and ecological considerations. Malaria caused by *Plasmodium faciparum* is the most serious type because it often proves fatal unless prompt treatment is given. *P.falciparum* resistance to clinically used drugs such as chloroquin, mefloquineetc is a serious problem and in parts of South East Asia and Africa, the only effective antimalarial drug is artemisinin or one of its derivatives. In many tropical countries extensive list of plants useful for the treatment of malaria is available. The ethnomedical information of *Tectonagrandis* reveals that it is one among them.

Reason for selection of this plant:

It was reported that Verbenaceaefamily members have phytoconstituents with various pharmacological properties useful in the treatment of bacterial, fungal, malaria and

pain arising from various causes including rheumatoid arthritis. It was also claimed that these plants merit detailed study which can prove useful in the discovery of lead compounds leading to novel and more efficacious drugs. [Rahmatullah Md.,2011]

Tectonagrandis belonging to the family Verbenaceaereallydo not have any match as a cheap natural and easily available plant. Its leaves traditionally known to be useful for the treatment of wide panel of diseases like tuberculosis, various kinds of **wounds** especially burn wound, **malaria, anaemia**, leprosy, skin diseases like pruritis, stomatitis, indolent ulcers, haemorrhages, menstrual disorder, bone joint disease. Young leaves and fruits prescribed for women for fertility control and fumes of dry leaves to kill guinea worms. It also possesses anti- ulcer and anti- cancer activity. Various scientific investigations of the leaves showed anti-diabetic activity, anti-microbial activity, anti-oxidant, analgesic, anti-inflammatory, anti-amoebic, anti-hypertensive and wound healing activity.

Bark is used as astringent, expectorant, anti-inflammatory, anthelmintic and for constipation, leucoderma, headache, piles, bronchitis, hyperacidity, and dysentery, burning sensation, diabetes, difficult in labour, leprosy, skin diseases, and indigestion. Researches proved it is anti-fungal, anti-bacterial, uterine relaxant, anti-histaminic, anti-asthmatic, anti-oxidant, analgesic and anti-inflammatory, anti-diabetic, hepatoprotective and cardiac activities.

Wood traditionally used as diuretic, stimulant, hepatic astringent, toothaches, sedative to gravid uterus, leucoderma. It was reported that wood possesses anti-oxidant, anti-termite, anti-hyperglycaemic, nephroprotective activities.

Flowers used for bronchitis, biliousness, urinary discharge, diuretic, depurative, anti-inflammatory, leprosy, skin diseases, diabetes, congestion of liver.

Seed is used in poisoning. Seed oil in skin disease. Oil obtained from seeds and flowers promote the growth of hair, eczema, ring worm, scabies. Scientific investigation of seed extract proved hair growth activity, diuretic, anti-inflammatory activity.

Roots are useful in anuria and retention of urine, anaemia. Anti-ulcer activity, anti-inflammatory, anti-tussive, anti-oxidant, hypoglycemic, anti-pyretic activities have been evaluated and proved.

It has been reported that bioflavones and flavonol glycosides were found to possess anti- malarial activity.[Khaomek P.,2008].

Betulinic acid is a lupane type triterpene that is widely distributed in the plant kingdom and this compound along with various derivatives has been shown to have anti-cancer, anti-bacterial, anti-malarial, anti-HIV, anthelmintic, anti-inflammatory and anti-oxidant properties.[Cichewicz RH and Kouzi SA .,2004, Yogeeswari P and Sriram D., 2005]

The survey of literature on *T.grandis*also reveals that leaves contain various phytoconstituents like flavonoids especially apigenin and steroidal compounds like betulinic acid, glycosides like anthraquinones etc.

Review of literature showed lacunae exist in the pharmacognostic, phytochemical and pharmacological studies. The present study assesses the potential of *T.grandis*leaves in relation to its traditional uses (in malaria) and in terms of findings on modern bio scientific research.

CHAPTER-2

LITERATURE SURVEY

VERBENACEAE

- Verbenaceaefamily plants used in Bangladesh was described. It was reported that they have constituents with various pharmacological property useful in the treatment of bacterial and fungal infection, malaria and pain arising from various causes including rheumatoid arthritis. The importance of plants used in Bangladesh lies more so in the potential for treatment of malaria and rheumatoid arthritis. It was claimed that the plants merit detailed studies which can prove useful in the discovery of lead compounds leading to novel and more efficacious drugs. [Rahmatullah Md. , 2011]
- Taxonomical classification of *T. grandis* plant[Aradhana R *et al.* , 2010]

Kingdom : Plantae

Subkingdom : Tracheobionta

Super division :Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Asteridae

Order : Lamiales

Family : Verbenaceae

Genus : *Tectona*

Species : *grandis*

Botanical Name :*Tectonagrandis.* Linn

Vernacular names for *T. grandis*:

Eng : Indian Teak

Hindi : Sagun, Sagwan

Mar : Sag, Saga, Sagwan

Guj : Saga, Sagach

Oriya :Singuru

Tel : Adaviteeku, peddateeku

Kan : Jadi, sagwani, tega, tyagadamara

Mal : Thekku, tekka

Tam : Tekkumaram, tekku [Anonymous. , 2005]

Cultivation History of *T. grandis*:

*T. grandis*natural distribution is in Southeast Asia, from the Indian subcontinent through Myanmar and Thailand to Laos. *T. grandis* early cultivation by Hindu settlers, possibly in the 7th century, is not clearly established. *T. grandis* is believed *T. grandis* was introduced to Java 400-600 years from India. At the beginning of this century, *T. grandis* was introduced to both East and West Africa and 'Trinidad *T. grandis*'has become particularly well known in the Caribbean region. *T. grandis* is planted for timber or ornament and in botanical gardens. *T. grandis* was the most important export timber in Thailand. [Aradhana R *et al.* ,2010]

Methods for cultivation:

The new plants can also be propagated from cuttings apart from the seed sowing. *T. grandis* usually planted when it is 4 to 6 weeks old. Plough the land thoroughly and levelit. The best season to plant the *T. grandis* is monsoon, most probably after the first shower. Carry out weeding operations regularly. *T. grandis* requires loamy soil rich in humus and having the right content of moisture with good drainage. *T. grandis* grows well in hilly and dry areas. *T. grandis* requires a dry tropical climate for its growth. *T. grandis* flowers in February and March. [Aradhana R *et al.* ,2010]

Natural Regeneration:

T. grandis naturally reproduces from seed but the extent of natural regeneration depends on climatic and edaphic factors. Fertile seeds begin to be produced on trees from near about the 20th year. Coppice shoots however, flower and fruit abnormally early, taking not more than 10 years to produce fertile seeds. The *T. grandis* produces seeds every year, poor seed years being occasional.

Factors influencing natural regeneration:

- Spread of the seed
- Germination
- Survival and development of the seedling

These factors almost possibilities for natural regeneration. The amount of regeneration increases with the increase in moisture and depth of the soil. The soil areas classified as, semi-moist *T. grandis* type, moist *T. grandis* type, very moist evergreen type.

Semi moist *T. grandis* type:

This type show less incidence of fire, but grazing continues to be heavy. Generally in this type, the ground cover is fair and natural regeneration of *T. grandis* is fairly adequate. The regeneration process, though slow, is steady and this tendency may be attributed to:

- Beneficial effect of mixed vegetation on soil
- Survival of*T. grandis* seedlings as a result of fire protection
- Moderate growth of bamboos, eliminating grass and other herbaceous growth of which *T. grandis* is intolerant
- Establishment of *T. grandis* seedlings under moderate shade of bamboos

- Protection of seedlings from desiccation by the under storey
- Relatively greater resistance of *T. grandis* seedlings to damage by cattle and fires as compared to the seedlings of its companions
- The capacity of seedlings to regenerate after cutting back, if sufficient overhead light is available.

Moist *T. grandis* type:

This type is better protected from fire and grazing is low. It appears that, before bamboos and other shrubby growth become dense and the upper canopy starts closing up, the conditions are most favourable for regeneration of*T. grandis* towards the drier end of this type. In the more humid parts, where the overwood is comparatively denser, canopy manipulation does bring in regeneration, but the progress is slow, and such areas cannot be relied upon for natural regeneration. The ground cover is dense and consequently natural regeneration occurs only in patches.

Very moist ever green type:

In this type prolonged fire protection results in the formation of dense understorey of semi ever green shrubs which inhibit regeneration of *T. grandis*. Consequently this type is particularly devoid of natural regeneration and the percentage of *T. grandis* is generally quite slow. [Anonymous. ,2005]

Artificial Regeneration:

T. grandis has been made to propagate by vegetative methods. Cleft grafting, budding and branch cuttings have been successfully employed for establishing clones of desired quality. Almost 80-100% success has been obtained in case of cleft grafting and

bud grafting but in branch cuttings only 60% success has been recorded. Other methods like air-layering etc. .were not successful in this plant. [Anonymous. ,2004]

Propagation:

T. grandis is propagated mainly from seeds. Germination of the seeds is often poor and sporadic. It involves pre-treatment to remove dormancy arising from the thick pericarp. Pre-treatment involves alternate wetting and drying of the seed. *T. grandis* seeds are soaked in water for 12 hours and then spread to dry in the sun for 12 hours. This method is repeated for 10-14 days. Dry heat method is better for drying. Seeds are heated for 1-5 weeks at 50˚C or at 48 hour at 80˚C. Then the seeds are sown in shallow germination beds of coarse peat covered by sand. *T. grandis* seeds are germinate after 15 to 30 days. [Aradhana R *et al.* ,2010]

Collection of *T. grandis:*

In dry situations and seasons, *T. grandis* leaves fall from November to January, while in moist localities the tree may remain in leaf until March or even later. As a rule, *T. grandis* trees are leafless throughout the greater part of the hot season, the new leaves appear from April to June but in wet seasons they sprout early. *T. grandis* flowers come up from June to August or September but, like the leaves, they may begin to appear in April under abnormally wet conditions. *T. grandis* fruits ripen from November to January and fall gradually and may be collected from under *T. grandis* trees. For easy storage *T. grandis*fruits, The calyx is removed by filling the bag with fruits and vigorously rubbing and shaking it; the remains of the calyces can be separated from the nuts by winnowing. The *T. grandis* nuts vary in weight(Madhya Pradesh), the number of fruits varied from 2, 000 to 3, 000/kg. [Anonymous. ,2005]

Natural Habitat:

T. grandis will grow and survive under a wide range of climatic and edaphic conditions(warm, moist, tropical climate)with significant difference between dry and wet seasons. *T. grandis* is deciduous, while its grown in non-seasonal climates are semi-deciduous. *T. grandis* is often a dominant member of a mixed deciduous forest, where it is main associates are *Xyliaspp, Afzeliaxylocarpa, Terminaliaspp* and *Lagerstroemia spp.* The forest floor is often covered with Bamboo. It is generally occurs scattered but can form almost pure stands under suitable conditions. Young plants show a remarkable capability to recover after fire. [Aradhana R *et al.* ,2010]

Geographical Distribution of *T. grandis*:

T. grandis is native in India, Western peninsula, Konkani, Burma, Myanmur, Thailand and Laos and exotic to Antigua and Barbuda, Bangladesh, Barbados, Dominica, Dominican Republic, Ghana, Grenada, Jamaica, Kenya, Brazil, Cambodia, China, Brunei, Cote d'lvoire, Cuba, Guadeloupe, Malaysia, Mauritius, Nepal, Nigeria, Pakistan, Panama, Philippines, Puerto Rico, South Africa, Sri Lanka, St Lucia, St Vincent and the Grenadines, Tanzania, Togo, Trinidad and Tobago, Uganda, Vietnam, USA, Virgin Islands(US). [Anonymous. ,2005, Aradhana R *et al.* , 2010].

Provenances:

Several provenances of *T. grandis* differing in growth characteristics, morphological characters of stem, leaf and timber have been recognized. Five distinct ecotypes of *T. grandis*forests(very dry, dry, semi dry, moist and very moist) are found in India. Dry and very dry area timbers has good figure and is valued for panelling and furniture. *T. grandis* from western regions (Malabar) is superior in strength to that from drier areas and is practically equivalent to the *T. grandis* from Burma. Hudsa plantation of Virnoli Range in North Kanara was observed to have smaller leaves with smooth, shiny,

dorsal surface. This seems to be a mutant type and known as *Teli* type which is stronger, durable and much cleaner than the common *T. grandis*, more cylindrical with very little fluting with less lower branches, better germinating seeds, smooth bark and also lefing and flowering are in advance. It appears to be resistant to defoliation by *Hyblaeapuera*Cramer. Another provenances from Ramanathapuram and Tirunelveli districts is reported to have different phenology. It have leaves of smaller, elliptic-ovate with acuminate tip with silver-glaucous under surface. Based upon a study of morphological differences, especially of leaf, a key, prepared by Bor, distinguishes several Burmese and Indian provenances, including those of Nilambur and other parts of Kerala, Shimoga, Dhulia, Kamara and Jhansi. It has been recommended that for planting a particular site, seed from a local or nearby and similar climatic regions should be considered the safest, unless by provenances test, some other provenance has shown better performance than the local source of seed. (Anonymous. ,2005)

Climate:

T. grandis thrives best reaches its largest dimensions in a fairly moist, warm, tropical climate, although it occurs even in dry localities, subjected to great heat and drought during the hot season. In highly moist, tropical regions *T. grandis* tends to be replaced by evergreen species. *T. grandis* tree may extend into regions of slight frost, but throughout *T. grandis* distribution the frost is generally unknown. *T. grandis* thrives best in areas having a normal rainfall, varying from 125-250 cm, with a marked dry season of 3-5 months, but *T. grandis* is known to grow, though not adequately well, in places with a rainfall as low as 75 cm or even less(Dhulia, Jalgaon, Nimar, Buldana, Ahmadnagar and West Karnool)and as high as 500 cm(West Coast). In the Indian Peninsula, it experiences in places absolute maximum shade temperature of 48˚ and absolute minimum shade

temperature of 2˚ but these extremes denote a drier climate than is favourable to *T. grandis* tree development. In the moist parts of the West Coast, however, where *T. grandis* reaches larger dimensions as compared to the trees in the drier parts of the peninsula, the climate is much more equable, the absolute maximum shade-temperature varying from 39 to 44˚C and the absolute minimum from 13-17˚C. (Anonymous. ,2005)

Soil:

T. grandis prefers rich soils. *T. grandis* tree is quite hardy, but the extent to which *T. grandis* flourishes and the quality of timber not only depend on the physical nature of the soils, but also on their depth, drainage, moisture content and fertility. *T. grandis* shows very good development on the fertile lower slopes of the hills, where the soil is deep , but in shallow soils and along dry ridges *T. grandis* becomes stunted. *T. grandis* comes up on soils produced by a variety of geological formations like traps,basalt, granitic gneisses, calcareous crystalline rocks, phyllites and schists. *T. grandis* also grows well on easily disintegrable sandstones like Vindhyan sandstones, but on quartzite and hard, metamorphic sandstones growth is poor. The great trap areas of the peninsula are extensively covered with *T. grandis*, but the trees are small in size as the soil is of no great depth;in the valleys and along the lower slopes, where the soil is deep, the trees attain fair dimensions. Good growth is noticed on soil produced by rocks rich in calcium oxide and other bases. *T. grandis* also thrives well on freshly deposited river- alluvia, having sufficient contents of exchangeable calcium. *T. grandis* tree avoids laterite;only where the laterite is highly disintegrated and mixed with other rocks does *T. grandis* attain any size. *T. Grandis*is not found on water logged ground or sites liable to prolonged inundation nor does *T. grandis*grow on stiff clay, black cotton soils and deep dry sands. Good growth of *T. grandis* is also not generally found on Gondwana rocks of plateau region. Rocks yielding

coarse-textured, nutrient-deficient, shallow soils are not good for *T. grandis* growth. *T. grandis* was found that soils having a lower silica sesquioxide ratio, lower dispersion coefficient and a very low or very high watertable are unfavourable for good growth of *T. grandis*. The root being very sensitive to the deficiency of oxygen, *T. grandis* does not withstand water-logging and requires well-aerated soils. The pH of soils in the *T. grandis* forests is between 5. 0 and 7.0, reaching 7.5 in drier tracts.In Madhya Pradesh the soils having more than 0.39% exchangeable calcium, support the growth of *T. grandis* and also good natural regeneration of soils containing 5-7mg phosphorus/100gm. [Anonymous. ,2005]

ELEMENTAL DEFICIENCY

Nitrogen:

It causes stunted growth, strong chlorosis, premature defoliation and absence of branching in seedlings.

Phosphorus:

It causes the seedlings exhibit scorched leaf margins and chlorosis, followed by necrosis, wrinkled leaf surface with restricted shoot growth and absence of branching.

Potassium:

In seedlings, it causes intervenalchlorosis and scorching of margins. In younger leaves the surface wrinkles and margins curl inwards.

Calcium:

In seedlings, is exhibited by severe intervenalchlorosis, distortion of leaves, and premature defoliation with restricted branching. [Anonymous. ,2005]

Diseases:

Oliveatectonae(rust stem and leaf); *Phyllactiniaguttata*and *Uncinulatectonae*(mildew); *Cercosporatectonae*(leaf spot); *Nectriahaematococca*(stem canker); *Corticiumsalmonicolor*(pink disease); *Phomopsistectonae*in combination with *Colletotrichumgloeosporioides*(leaf spots); *Pseudomonas tectonae*and *P. tectonae*(wilt); *Fusariumoxyporum*(damp. off); *Armillariellamellea*(root rot); *Helicobasidiumcompactum, Phellinusnoxius, Rigidoporuslignous, R. zonalis*and*Peniophorarhizomorpho-sulphurea*(butt rot); *Pellinusnoxius, P. lamaoensis, Ustulinadeusta, Polyporusrubidus, Ganodermaapplanatum*and *R. zonalis*(heart rots); *Cossuscadambae*(trunk borer); *Phialophorarichardsiae*(die-back of trees); *Dendrophthoefalcata*(the mistletoe) insects*Holotrichia spp.* (white grubs); *Sahyadrassusmalabaricus*(sapling borer); *Cossuscadambae*(trunk borer); *Hyblaeapuera*(defoliators*)*; *Eutectonamachaeralis, Pyraustamachaeralis, Hapaliamachaeralis*and *Eutectonamachaeralis*(skeletonizer*); Xyleutesceramica*(bee hole borer and shoot borer); *Duomitusceramicus*(bee hole borer); *Zouzeracoffeae*(shoot borer);*Pagidasalvaris*; *Machaerotaelegans; Mylabrisphalerata;Dichocrosispunctiferalis; Eublemma spp. ; Xyleborusdestructuens;Calotermestectonae;X. padestruens and Coptotermeselisae (*termites*)Pellicullariasalmonicolor*(bark flaking)*Peniophorarhizomorpho-sulphurea* causes serious mortality due to rootrot in plantations in North India. The fungus produces subterranean rhizomorphs through which the disease spreads. *Polyporuszonalis* causes root and buttrot in plantations, In dry coppice *T. grandis* forests, 50%trees exhibit hollowness due to decay of heartwood by *Polyporuszonalis* and *Fomeslividus* at the end of rotation. Heartrot is present in the callus shoots arising from cambial activity at the cut end of stools; as these shoots develop intimate organic connection with the stools during their growth, the fungi migrate from the stools to the heartwood of shoots. However, coppice shoots arising from dormant buds at or below the ground level, develop their own root system and in due

course become independent of stools and thus remain free from heartrot. [Anonymous. ,2005]

LEAVES:

ETHNOMEDICAL INFORMATION:

- The young leaves and fruits are prescribed to women for fertility control by the herbal vendors. In Rajasthan, the fumes of dry leaves are used to kill guinea worms in infested patients. [Jayakumar*et al.* , 1987]
- *T. grandis* have revealed that the plant has anti-ulcer [Pandey *et al.* , 1982], anti-microbial [Sumthong*et. al.* , 2006], anti-cancer[Khan RM and Miungwana SM. , 1999]
- Leaves are used for stomachic, astringent and vermifuge.
  [Kamat SV. ,2001]
- Leaves have also been used in indigenous medicine. Extracts of the leaves showed complete inhibition of *Mycobacterium tuberculosis* [Anonymous. , 2005]
- Tumours were treated with leaves of *F. glomerata*, *T. grandis* and *E. scaber* repeatedly and then with a honey mixed fine paste of *A. roxburghiana, C. sappa, S. racemosa, T. arjuna, X. strumarium* was applied. [Premalatha and Rajgopal. , 2005]
- The leaves of *T. grandis* are widely used in India folklore for the treatment of various kinds of wounds especially burn wound [Manoharan KP *et al.* , 2007, Majumdar M *et. al.* , 2007] Leaves of *T. grandis* is used as anti-inflammatory and topical treatment for burn injuries like burn inflicted wounds and skin ulcers. [Yogesh Sharma *et al.* , 2013]
- The leaves of *T. grandis* with or without *J. curcas* and *F. flavenscens* are traditionally used in Togo to treat malaria and other anaemia diseases. [Diallo A *et al.* , 2008]

- *T. grandis* leaves used for cooling, haemostatic, depurative, anti-inflammatory and vulnerary, leprosy, skin diseases, pruritis, stomatitis, indolent ulcers, haemorrhages, haemoptysis, vitiated conditions of pitta. [Aradhana R *et al.* , 2010, Khera N and Bhargava S. , 2013]
- *T. grandis* leaf, root, flower usedfor constipation, blood dysentery, skin diseases, powdered roots and boiled leaves are orally administered for constipation and blood dysentery, macerated leaves and flowers are applied to affected areas in skin diseases. [SajjadulkarimMd*et al.* , 2011]
- Decoction of *T. grandis* fresh or dried leaves are used for menstrual disorder and haemorrhages[Kothale KV *et al.* , 2012]
- *T. grandis* leaves used as remedy for bone joint disease by valaiyar community in Madurai district. [Palaniappan P *et al.* , 2012]
- *T. grandis* young leaves, bark, wood traditionally used for fever, malaria, stimulant, anaemia, diabetes II, anti-ulcer, clothes industry of bits of matches. [Bangou MJ *et al.* , 2012]

PHARMACOGNOSTICAL STUDIES

- *T. grandis* leaves are elliptic or obovate, acute or acuminate, the upper surface rough but usually glabrous, the lower clothed with dense stellate grey or tawny tomentum, entire, base usually cuneate, main nerves 8-10 pairs with 2 or 3 large branches near the edge of the leaf, joined by numerous parallel transverse veins. [Kiritikar Basu. , 1987]
- *T. grandis* leaves was studied for the drug weight loss and chemical changes (decomposition process) in a small fresh water lake using plastic net bags. (1mm pore size). The results revealed that dry weight loss and changes in the levels ofcellulose, hemicellulose, lignin, sugar, amino acid and nitrogen of *T. grandis*

leaves at different depths in a shallow freshwater lake. The rate of decomposition was faster when compared with similar studies in Canada and Europe. [Tiwari BK and Mishra RR. , 1983]

- The leaf extract of fallen leaves showed inhibitory effect on the growth of *Arachis hypogea and Zea mays.* Chlorogenic, coumaric and ferulic acids present in the extract are probably responsible for the allelopathic effects. [Anonymous. ,2004] .
- Naphthotectone showed high level of activity(aqueous solution concentration ranging from 10^{-3} to 10^{-5} M) in etiolated wheat coleoptile bio assay as well as standard target species lettuce, cress, tomato and onion suggested that it may be involved in the allelopathic activity and in other defence mechanism their by this makes naphthotectone and important future target in the development of drugs and eco herbicides. [Lacret R *et al.* , 2011]
- Structures of isolated compounds from *T. grandis* leaves were determined and studied (using etiolated wheat coleoptiles). The activities showed that the isolated lignans and nolignans should be part of the defence mechanisms of this plant. [Lacret R *et al.* , 2012]

PHYTOCHEMICAL STUDIES

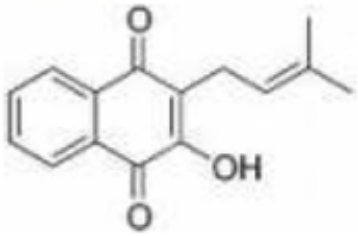
- A novel compound tectograndone derived from the interaction of two prenylatednaphthoquinones has been isolated from the leaves of *T. grandis*. Thestructure was elucidated as 10-(2, 5, 8-trihydroxy naphthaquinone-3yl)-5, 12-dihydroxy-2, 2, 8 trimethyl-2(H)-pyrano(2, 3, -b)anthracene-6, 11-dione, primarily by 1D and 2D NMR techniques. [Aguinaldo *et al.* , 1993]

- Leaves are containing a yellow or red dye which has been used and strongly recommended for dyeing silk yellow olive or related shades. Crushed leaves are produce a red colour when rubbed with saliva [Anonymous. 2005]

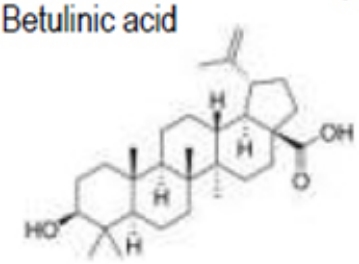
- *T. grandis* leaf reported to contain carbohydrates, alkaloids, tannins, sterols, saponins, proteins, calcium, phosphorus, crude fibre and dyes(yellowish-brown or reddish). [Anonymous . , 2006, Krishnan KS. , 2006, Majumdar M *et al.* , 2007, Goswami DV *et al.* , 2009, Nayeem N Karvekar . , 2010]
- A new Anthraquinonetectone(1), 14 known compounds (2-5)[5 terpenoids2-5, 15], 4flavanoids(6-9), 3 flavone glycosides(10-12), 2 phenolic glycosides(13-14)were isolated and identified synthesized from the chloroform and n-butanol fractions of ethanolic extract of *T. grandis* leaves. [Shukla N *et al.* , 2010]
- *T. grandis* leaves contain reported chemical constituents are

- Quinones(tectoquinone, lapachol, deoxylapachol and its isomer, tectoleafoquinone, anthraquinone-napthaquinone pigment)
- Steroidal compounds(squalene, poly isoprene-α-tolyl methyl ether, **betulinic acid,**tectograndone, monoterpene, Apocarotenoids: Tectoionols A, Tectoionols B.
- Glycosides (Anthraquinone glycosides)
- Phenolic acids(tannic acid, gallic acid, ferulic acid, caffeic acid and ellagic acid).
- Flavonoids (rutin and quercetin). [Aradhana R *et al.* , 2010]

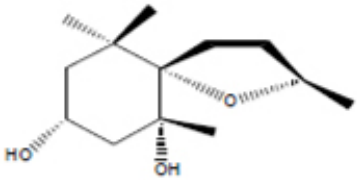
Structure of quinone
Lapachol



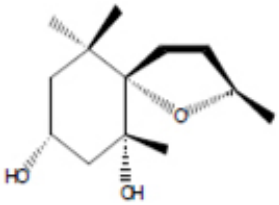
Structure of steroidal compound
Betulinic acid



Structure of Apocarotenoids:
Tectoionols-A

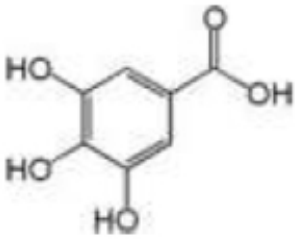


Tectoionols-B

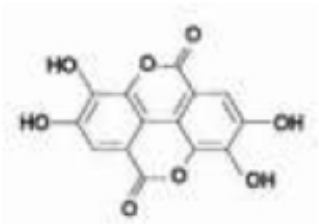


Structures of Phenolic Acids

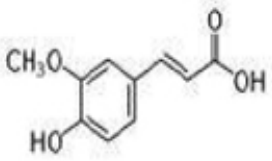
Gallic acid



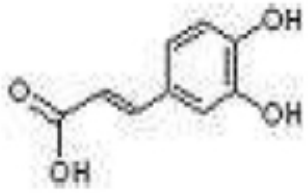
Ellagic acid



Ferulic acid

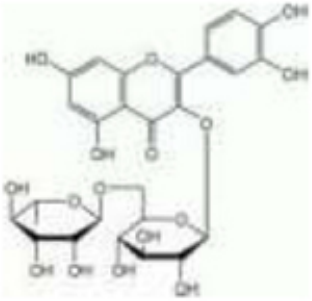


Caffeic acid

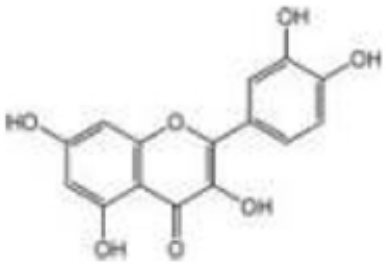


Structures of Flavonoids

Rutin



Quercetin



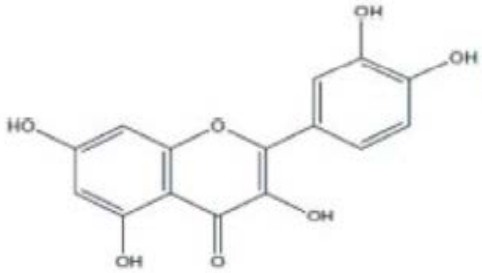
- A comparative phytochemical analysis of methanolic extract of frontal and mature leaves of *T. grandis* were showed that the total phenolic and flavonoid content was more in the frontal leaf extract than that of matured leave[Nayeem N and Karvekar MD. , 2010][a]
- The presence of gallicacid, ellagicacid, rutin, quercitin, identified and isolated from methanolic extract of the frontal leaves of *T. grandis*.

  [Nayeem N and Karvekar MD. , 2010][b]
- An isoprenoidquinone and dimericanthraquinone, two new quinones named as naphthotectone and anthratectone respectively from bio-active extract of *T. grandis*. [Lacret R*et al.* , 2011]
- Chloroform extract of *T. grandis* leaves isolated active ingredients of anthraquinones, naphthoquinones, juglone, lapachol and deoxylapachol, emodin, parietinic acid possessed potent anti-bacterial activity[Mahesh S Krishna, Jayakumaran Nair A. , 2011]
- The compounds gallicacid, rutin, quercitin, ellagicacidandsitosterol were isolated from the methanolic leaf extract of *T. grandis*. [Nayeem N and Karvekar MD. , 2011][a]
- Preliminary phytochemical screening showed the presence of saponins, carbohydrates, cardiacglycosides, steroids, triterpenes. Alkaloids, tannins, phenols, flavonoids, proteins, aminoacids, fats and oil found to be absent. Extractive value found to be as follows by soxhlet petroleum ether:6. 5, chloroform:6. 9, ethanol:14. 6, aqueous:27. 14(maceration). [Pradeep G *et al.* , 2012]

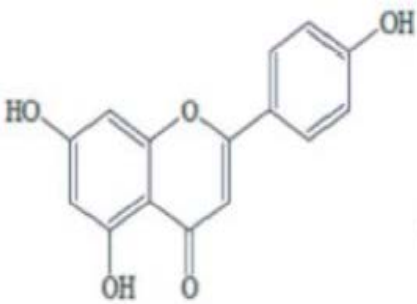
Cardiac glycosides are found to be present in *T .grandis.* Alkaloids shown positive results with Dragendroff's reagent in *T. grandis*leaves. Anthraquinones, flavonoid, leucoanthocyanin present only in *T. grandis* leaves. [Kothale KV *et al.* , 2012]

- It was reported that the methanolic extract of *T. grandis* leaves found to contain more flavonoids and also contain alkaloids, tannins, anthraquinones, naphthaquinones. [JangameCM and Burande MD. , 2013]
- Ethyl acetate and n-butanol fractions of *T. grandis* leaves were isolated(2 phenolic acids and 8 flavanoids) and purified by different column chromatography. The isolated compounds were elucidated(UV, IR, NMR, Co-PC, Co-TLC and Co-mp) and identified (acid hydrolysis) as quercetin, apigenin, gallic acid, quercetrin, chlorogenic acid, diosmin, kaempferol, toxifolin and hesperidin. [Ghareeb MA *et al.* , 2013]

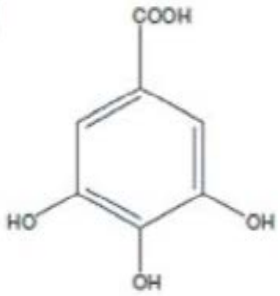
Quercetin



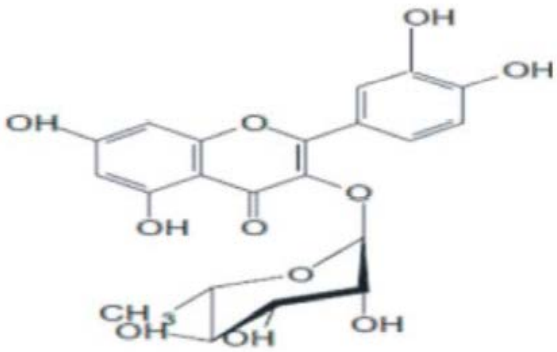
Apigenin



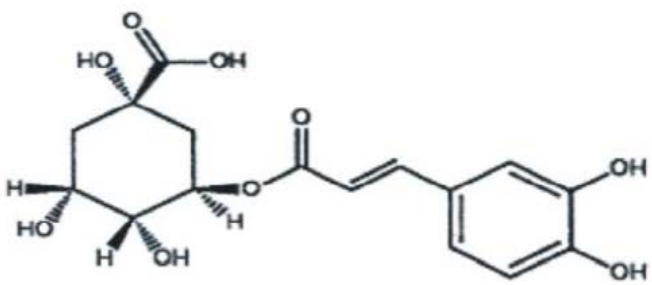
gallic acid



Quercetrin

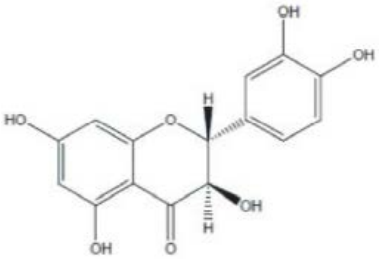


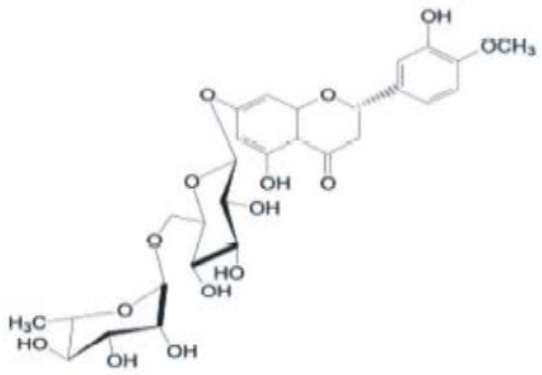
Chlorogenic acid



diosmin

Taxifolin hesperidin





- Defatted 90% methanol leaf extract of *T. grandis* showed the presence of alkaloids, flavonoids, saponins, terpenoids, steroid, tannins, anthraquinones, coumarins and sterols. [Ghareeb MA *et al.* , 2014]

PHARMACOLOGICAL STUDIES

ANTI DIABETIC ACTIVITY

- Chloroform and n-butanol fractions of the ethanolicextractof*T. grandis* leaves compounds(tectone, 2 terpenoids, phenolic glycoside)showed significantanti-hyperglycemic activity(100/kg/bw)in STZ induced diabeticrats, when compared to metformin. [Shukla N *et al.* , 2010]
- Significant anti-diabetic activity on glucose tolerance in alloxan induced diabetic rats was reported at 30^{th}mts for ethanolic extract(250mg/kg P. O). [Pradeep G *et al.* , 2012]

CYTOTOXIC ACTIVITY

- Against chick embryo fibroblast cells hexane extract of *T. grandis* leaves showed 54% inhibition(but chloroform bark extract showed 87%)[Mahesh S Krishna and Jeyakumaran Nair A. , 2010]

- *T. grandis* leaf extracts(defatted 90%methanol, n-BuOH, EtOAc)was evaluated using preliminary brine shrimp and towards liver cancer cell line(Hep G_2 using Sulphorhodamine B assay). *T. grandis* leaf extracts of n-butanol(IC_{50}=11. 6µg/mL), 90% defatted methanol(IC_{50}=19. 7 µg/mL), ethyl acetate(IC_{50}=22%µg/mL)showed high cytotoxic activity toward the Hep G_2cell line comparing with the standard doxorubicin(IC_{50}=4µg/Ml). [Ghareeb MA *et al.* , 2014]

ANTI MICROBIAL ACTIVITY

- Anti-bacterial activity of *T. grandis* leaf extracts(hexane, methanol, ethylacetate, chloroform)were evaluated against *S. aureus, K. pneumoniae, S. paratyphi* and *Proteus mirabilis*(disc diffusion assay). Chloroform leaf extract showed inhibition to growth of *S. aureus*(14mm)and *K. pneumoniae*(8mm). [Mahesh S Krishna andJeyakumaran Nair A. , 2010]
- The methanolic extract of *T. grandis* leaves were examined (MIC&MBC)anti-bacterial activity(disc diffusion method). Leaf extract showed anti-bacterial activityagainst *Streptococcusspecies*(Grampositive), *K. pneumoniae, P. aeruginosa, P. mirabilis, E. coli, S. typhimurium*(Gram negative).

  Methanolic extract of *T. grandis* leaves were alsoevaluated(MFC) foranti-fungal assay(disc diffusion method). Leaf extract showed significant activity against fungal strains of *C. albicans, C. neoformans, A. niger, A. flavus, A. fumigatus, Rhizopussp*(*T. rubrum, T. gypseum, T. mentagrophytes*)[Purushotham KG *et al.* , 2010][a]
- Methanolic extract of *T. grandis* leaves showed maximum synergitic activity against different gram positive and gram negative bacteria along with tetracycline. The highest synergitic rate was against *S. typhimurium*(MTCC

98)*Klebsiellapneumoniae*(MTCC 432)and lowest action against*Pichiapastoris*(MTCC 34)*E. coli*(MTCC 729)and no synergitic activity against *Citrobacterfreondii*(MTCC 1658). [Purushotham KG *et al. ,* 2010][b]

- Anti-microbial activity of aqueous leaf extract *T. grandis* was tested (MIC & MBC) against *S. typhimurium, S. typhi, P. aeruginosa* (macrobroth dilution method). Aqueous leaf extract showed inhibition against *S. typhi* ranging from 2. 5to 5mg/ml(MBC). The results support for the use of *T. grandis* in traditional medicine for treatment of typhoid fever.

  [Bolou GEK *et al.* ,2011]

- Rutin isolated from the methanolic leaf extract showed significant anti-microbial activity against *S. aureus, B. substilis, E. coli, K. pneumoniae*(Gram positive and Gram negative)and fungi *C. albicans*. It was suggested that the activity was attributed to that number and position of phenolic-OH groups present. [Nayeem N and Karvekar MD. ,2011][b].

- Leaf extracts of*T. grandis*(ethanol, petroleumether, chloroform, aqueous)showed *in-vitro* anti-microbial activity against *E. coli* but maximal activity in methanolic extract. [Pradeep G et al . , 2012]

- *T. grandis*methanolic leaf extracts 0. 5%, 1%, 2% and 4%(w/v) were evaluated anti-fungal activity against *A. phaeospermum* causing wood decay(diffusion method on potato dextrose agar). The leaf extract at a concentration as low as 0. 5%(w/v)suppressed significantly the growth of *A. phaeospermum* (81. 4%w/v) with MIC (0. 4%w/v. The leaf extract inhibited significantly the fungal radial growth, the total bio mass and sporulation. [Ni Putu and Dewa Ngurah. , 2012]

ANTI OXIDANT ACTIVITY

- Hexane, methanol, ethylacetate, chloroform extract of *T. grandis* leaf showed anti-oxidant activity. [Mahesh S Krishna, Jeyakumaran Nair A. , 2010]
- Quercetin isolated from the methanolic leaf extract showed significant anti-oxidant activity byDPPH method (IC_{50} 60. 38). The decreasing order of activity was as follows quercetin>rutin>gallic acid>ellagic acid>sitosterol. [Nayeem N and Karvekar MD. , 2011]
- Different leaf extracts of *T. grandis*(petroleum ether , $CHCl_3$, Ethyl acetate and n-butanol)was evaluated by three assays(DPPH, Phosphomolybdenum, RPAA). *T. grandis* leaf extract showed strong antioxidant activity when compared with standard(L-ascorbic acid). [Ghareeb MA *et al.* , 2014]

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY

- The methanolic extract of the frontal leaves of *T. grandis* showed dose dependent(250mg/kg bw)analgesic activity at 15, 30, 60, 120mts(Eddy's hot plate method)and significant anti-inflammatory activity after 15mts better than that of the standard(carrageenan induced paw edema method). The researches claimed the action is due to the presence of poly phenolic compounds like phenolic acids, flavonoids and tannins. [NayeemN, Karvekar MD 2010][c]
- The methanolic extract of *T. grandis* frontal leaves(250 mg/kg bw) and mature leaves (500 mg/kg bw)showed analgesic activity at 15, 30, 60, 120 mts(Eddy's hot plate method)and significant anti-inflammatory activity after 15mts better than that of the standard (carrageenan induced paw edema method). The results of the frontal leaf

extract has shown better activity when compared to the mature leaves by virtue of the difference in the amount of phytoconstituents. [NayeemNandKarvekar MD. , 2012]

ANTI-ANAEMIC ACTIVITY

- Oral administration of *T. grandis* extract at 1gm/kg/day and 2gm/kg/day to the rats previously treated with phenylhydrazine, increased the concentration of haemoglobin, red blood cells number, haemoctocrit and reticulocytes rate. Moreover, the extract of *T. grandis* enhanced the osmotic resistance of the red blood cells that confirm the important presence of young red blood cells. These results support partially the traditional use of *T. grandis* in the treatment anaemia. [Diallo A*et al. ,* 2008]

ANTI-HYPERTENSIVE ACTIVITY

- Ethanolic extract of *T. grandis* leaves has significantly decreased (p<0. 05)blood pressure of renal artery occluded hypertensive rats at the dose levels of 20, 40, 80 mg/kg/I. V and captopril showed (p<0. 05)elevated blood pressure at the dose of 1mg/kg. This result would justify the traditional use of the herb for the management hypertension and thus may be due to the action on renin angiotension system. [Ajay GO *et al. ,* 2011]

WOUND HEALING ACTTVITY

- Oral administration of *T. grandis* leaf extract showed wound healing activity (incision wounds, &excision wounds)in wistar rats when compared with control. *T. grandis* leaf extract increased the tensile strength, decreased the epithelisation period and showed faster rate of wound contraction as compared to control. [Upadhya VK *et al. ,* 2000]
- The hydrochloric extract of *T. grandis* leaves on excision, incision, burn, dead space wound showed; significant reduction in period of epithelisation and wound

contraction(50%)[excision], a significant increase in the breaking strength[incision]topical(5%&10%), significant increase in the breaking strength, dry weight hydroxyl proline content of the granulation tissue by oral route(250&500 mg/kg bw)[dead space wound]. It was concluded that it possesses wound healing activity and support folklore claim. [Majumdar M *et al.* , 2007]

- Comparative study of wound healing activity in excision wound model using methanolic extract of frontal and mature leaves *T. grandis* showed significant activity which was 100% on the 12^{th} day and 16^{th} day in the frontal leaves ($p<0.001$)and the mature leaves ($p<0.05$)respectively. This support the ethnopharmacological claim that only the frontal leaves of *T. grandis* have been used for treatment of wounds. It was further proposed that more total phenolic and flavonoid content of frontal leaf may be the reason for this difference. [Nayeem N and Karvekar MD . , 2010][a]
- 0.2% ointment formulated using the isolated constituents are gallicacid, rutin, quercetin, ellagicacid, sitosterol in emulsifying ointment base showed complete wound healing from the 10^{th} day in the following order rutin>gallic acid >ellagic acid >sitosterol>quercetin in excision , incision rat wound model. Stability study of the ointment containing rutin was reported. [Nayeem N and Karvekar MD . , 2011]
- *T. grandis* leaf extract applied topically or given orally promoted the breaking strength, woundcontraction, collegenation. [Fatima S *et al.* , 2001, Yogesh Sharma *et al.* , 2013]

BARK

ETHNOMEDICAL INFORMATION

- Bark is used as astringent, constipation, anthelmintic, leucoderma, headache, piles, laxative, expectorant, anti-inflammatory. [Qudhia P. ,][Sharma PV *et al.* ,1986]and depurative. It is used in bronchitis, hyperacidity, dysentery, verminosis,

burningsensation, diabetes, difficult in labour[Shiddamallayya N *et al.* , 2008], leprosy and skin diseases[Ghasias M *et al.* , 2009], indigestion, expels worms from the body and in vitiated conditions of pitta[Khera N and Bhargava S. , 2013].

- The stem bark of *T. grandis* is made in to a paste with water ½ tumbler(above 50 ml)and given orally for relieving constipation daily one time by Irulas and Paniyas of Nilgiri District. [Rajan S *et al.* , 2001]

PHYTOCHEMICAL STUDIES

- *T. grandis* bark was found to containlapachonone& a naphthoquinone (lapachol). [Varma SB and Jaybhaye DL. , 2010]
- Phytoscreening of the *T. grandis* bark revealed the presence of flavonoids, tannins, glycosides and carbohydrates and also reported that extractive value of petroleum ether, ethylacetate, aqueousextract were found to be 2. 81, 6. 48, 5. 19% respectively. [Goswami DV *et al.* , 2010]
- Preliminary screening of stem bark of *T. grandis* revealed the presence of alkaloids, carbohydrates, cardiacglycosides, saponinglycosides, steroids, tannins, proteins and amino acids, flavonoids in ethanol and aqueous extract. [Asif Md. , 2011]
- Quinones and sterols have been reported in the ethanolic extract of *T. grandis* bark[Bagalli. , 2011]
- 70% alcohol extract of stem bark of *T. grandis* has shown the presence of carbohydrates, proteins, steroids, anthraquinoneglycosides, phenoliccompounds and saponins while glycosides, steroids and proteins are absent in aqueous extract. [Gaikwad*et al.* , 2011]
- A phytochemical study on bio-active extract from *T. grandis* was isolated two new nor-lignans(tectonoelinA, tectonoelin B)with ten known compounds[six lignans(5-10), 4

phenolic compounds(1-4)]. Structures of isolated compounds were determined and studied (using etiolated wheat coleoptiles). The activities showed that the isolated lignans and norlignans should be part of the defence mechanisms of this plant. [Macias *et al.* , 2008], [Lacret R*et al.* , 2012]

PHARMACOLOGICAL ACTIVITY

ANTI-FUNGAL AND ANTI-BACTERIAL

- The stem of reforestation specimen and fractionation of its hexane extract by classical procedures led to isolation of naphthaquinones, lapacholanddehydro-α lapachone and anthraquinones, tectoquinona and obtusifolina. It was also reported the presence of a substance belonging to the class of anthraquinones. Thetectoquinona (1)which are assayed anti-fungal, anti-bacterial and repellant attacks and some insects and is therefore liable the durability of wood and export to the rigors. [Rafael YOM *et al.* , 2006]

UTERINE RELAXANT ACTIVITY

- *T. grandis* bark (70%w/v methanol extract)has showed uterine relaxant activity in dose dependent manner and complete inhibition of contraction noted 32mg ($p<0.001$) comparable to standard uterine relaxant i. e Injection of Magnesium sulphate (75 mg), Nifidipine(0. 18 mg), Isoxsuprine (0. 18 mg). It was suggested that mechanism due to the inhibition of prostaglandin bio-synthesis. [Jaybhaye D *et al.* , 2010]

ACUTE TOXICITY

- No toxicity found in ethanolic extract of *T. grandis*bark(72 hrs)[Varma SBandJaybhaye DL. , 2010]

- The acute toxicity study for petroleum ether, ethanol, ethyl acetate using swiss albino mice as per OECD guidelines were found safe upto the dose of 2000 mg/kg for all extracts. [Goswami DV et al . , 2010]
- Acute toxicity study did not reveal any toxic symptom or mortality upto the dose of 2gm/kg bw both ethanol and aqueous extract of *T. grandis*(TGEE&TGAE). [Asif Md. , 2011]
- There was no lethality toxicity reaction found upto the dose of 6. 4gm/kg bw. [Bera S *et al.* , 2011]

ANTI HISTAMINIC ACTIVITY

- Ethyl acetate extract of *T. grandis* bark possesses anti-histaminic activity, inhibited clonidine induced catalepsy on not inhibited haloperidol induced catalepsy (100 mg/kg)in swiss albino mice model than that of petroleum ether&ethanol extract. [Goswami DV *et al.* , 2010]

ANTI-ASTHMATIC ACTIVITY

- Ethyl acetate extract of *T. grandis* bark possesses significant ($p<0.001$)anti-asthmatic activity compare to petroleum ether and ethanolic extract at a dose of 100mg/kg(P. O) and it was suggested that it may be attributed to mast cell stabilizing adaptogenic activity suggestive of its potential use in the treatment and prophylaxis of asthma. [Goswami DV *et al.* , 2010]

CYTOTOXIC ACTIVITY

- *T. grandis* bark extracts (hexane, methanol, ethyl acetate, chloroform)were checked for cytotoxic potential (MTT assay). Chloroform bark extract showed very high inhibition

activity against chick embryo fibroblast (87%)and human embryonic kidney cells(95. 3%). [Mahesh S Krishna and Jeyakumaran Nair A. 2010]

ANTI-BACTERIAL ACTIVITY

- Different extracts (hexane, methanol, ethylacetate, chloroform)of*T. grandis* bark showed anti-bacterial activity. [Mahesh S Krishna andJeyakumaran Nair A. 2010]

ANTI-OXIDANT ACTIVITY

- Ethyl acetate extract of *T. grandis* bark showed anti-oxidant activity against DPPH and $ABTS^{+}$free radicals. [Mahesh S Krishna andJeyakumaran Nair A. 2010]

ANALGESIC AND ANTI-INFLAMMATORY

- Both ethanol(TGEE) and aqueous(TGAE)extract of *T. grandis* showed significant($p>0.001$)dose dependent(200, 300, 500mg/kgp. o)analgesic and anti-inflammatory effect in wistar rats with hot plate test and carrageenan induced paw edema model. [Asif Md. , 2011]

ANTI-DIABETIC ANTI-OXIDANT ACTIVITY

- Oral administration of ethanol extract of *T. grandis*bark(2. 5 &5g/kg bw)showed anti-hyperglycemic effect in alloxan induced diabetic rats. Significant activity showed within 30days(250±6. 5 to50±2. 5mg/dL). These results support the folk medicine of *T. grandis* used as anti-hyperglycemic agent[Varma SB&Jaybhaye DL. , 2010]
- The methanolic extract of bark of *T. grandis* showed significant reduction in elevated plasma level along with decrease in serum creatinine, urinealbumin, urine total protein and significant increase in serum albumin, total protein and %body weight and showed absence of sclerotic lesion showed by diabeticconditioninalloxan induced diabeticrats,

henceindicate its potential to treat diabetes mellitus prevent associate the renal damage. [Ghaisas M *et al.* , 2010]

- The hydro methanolic extract of *T. grandis* bark recovered FBG activities of carbohydrate metabolic enzymes like G_6P, G_6P dehydrogenase, hexokinase in liver along with quantity of glycogen in the liver and skeletal muscle significantly($p>0.05$). A significant recovery also reported in the activities of anti-oxidant enzymes in catalysedperoxidase, TBR, substances in liver and kidney and hence it was indicated that the hydromethanolic extract of bark possess anti-hyperglycemic and oxidative stressin STZ diabetic rats(400mg/kg bw)Glybenclamide used as standard. [Bera S *et al.* , 2011]
- Ethanolic extract of the *T. grandis* bark showed potent anti-oxidant activity. Acute oral toxicity study showed 2gm/kg bwp. o. It showed significant anti-diabetic activity in alloxan induced diabetic rats in single and multiple dose study. Regeneration of islets of Langerhans and β cells were observed in histopathological examination of the pancreas(200mg/kgp. o). [BagalliRS . , 2011]
- The ethanolic extract of the bark of *T. grandis* showed significant anti-diabetic effect in alloxan induced diabetic rats (50, 100, 200mg/kg bwp. o)with significant decrease in bio-chemical parameters, atherogenic index and haemodynamic parameters were reported prevention of cardiac dysfunction alloxan induced rats. [Ghaisas M *et al.* , 2011]
- Methanolic extract of*T. grandis* bark altered parameters (blood glucose, glycosylatedhaemoglobin, protein, totalcholesterol, urea, serumcreatinine, Aspartatetransaminase, Alaninetransaminase, Lactate dehydrogenase, thiobarbituric

acid reactive substance, superoxidedismutase, catalase and glucose 6 phosphatase) were significantly controlled in the dose level of 300mg/kg bwin alloxan induced albino diabetic rats and hence claim to be potent anti-oxidant and anti-diabetic. [Rajaram Ke. , 2013]

ANTI- MITOTIC ACTIVITY

- While 70% alcohol extract of stem bark of *T. grandis* was subjected to column chromatography. It was reported that chloroform fraction was found to be the most anti-mitotic activity *Allium cepa*method and*brine shrimp lethality* test using*Artemia Salina* eggs than that of the aqueous extract. [Gaikwad*et al. ,* 2011]

HEPATOPROTECTIVE ACTIVITY

- Ethanolic extract of bark of *T. grandis* showed significant hepatoprotective activity in CCl_4treated rat(200mg/kg PO). [Ghaisas M *et al. ,* 2011]

CARDIAC ACTIVITY

- Oral administration of ethanol extract of *T. grandis* (50. 100, 200 mg/kg)stem bark showed significant effect on cardiac dysfunction in alloxan induced (single i. p administration)diabetic rats. Within 42 days *T. grandis* showed significant dose dependent decrease ($p<0.01$) in Bio-chemical, hemodynamic parameters(SAP, DAP, MAP)atherogenic index compared with diabetic control. The bark of *T. grandis* is traditionally used in the treatment of diabetes and associated with cardiovascular disease. [Ghaisas M*et al. ,* 2011]

WOOD

Teak wood is most important wood in this world. It is used for ship-building, heavy construction in general and bridge-building in particular, piles in harbours, railways and other coach-building, furniture and cabinet-making, flooring, joinery and general carpentry , poles and posts. Teak wood waste is used as fuel. [Anonymous. ,2005]

DISEASES:

Anthraquinone substituted compounds(lapachol, deoxylapachol and o-cresyl methyl ether) are resistance to termites. Toxicity of 4, 5-dihydroxy methyl anthraquinone resistance to *Cryptotermesbrevis* and *Coptotermeslacteus*.Wood extractives are against *Microcerotermesbeesonii*, *Heterotermesindicola* and *Polyporusversicolor*. Tectol, dehydrotectol and some naphthaquinones inhibit drying of polyester lacquers. Some constituents like lapachol have sensitizing effect and may cause allergic eczema or severe itching to some persons. Desoxylapachol is also dangerous. β-methyl anthraquinone act as synergist for DDT.

ETHNOMEDICAL INFORMATION

- Wood oil is for Ring Worm in Rajasthan &MP[Jain SK . , 2004]
- Paste made from the wood is used as adiuretic, stimulant, hepaticastringent, relief from toothache. Wood ash is applied to the swollen eyelids to strengthen the eye sight. Oily product obtained from the wood chips applied to eczema[Aradhana R *et al.* , 2010]
- Acrid, cooling, laxative, sedative to gravid uterus useful in treatment of piles, leucoderma and dysentery. Oil extracted from the wood is best for headache, bioliousness, buningpains, particularly over a region of liver[Khera N and Bhargava S. , 2013].

PHARMACOGNOSTIC STUDIES:

Timber of *T. grandis* have remarkable physical and mechanical properties, particularly shape-retention and durability. Teak is distinguished into two types on the basis of origin:

- Central Indian teak

It is darker(Madhya Pradesh)in shade and ornate in appearance, broad, wavy streaks.

- South Indian Teak

It is light coloured and more straight-grained.

Sap wood of teak:

It is white to pale-yellowish brown, the heart wood is golden yellow, darker streaks ,turning dark brown on exposure, moderately hard but rather coarse, uneven-textured and strongly odorous. It is medium heavy(sp. gr:0. 55-0. 70, wt:608-688 kg/cu. m), strong, and average hardness. Thewood is ringporous with the growth rings delimited distinctly by a lighter zone of large and clearly visible pores of spring wood and the darker zone of summer wood consisting of denser, fibrous tissue and smaller pores. Stronger wood have 2-5 growth rings/cm. The vessels are partly occluded with tyloses and occasionally filled with white and yellowish deposits. The rays are widely spaced, broad and distinct, conspicuous silvery flock on the radial surface. Teak is model wood for air-seasoning. It is stacked in open piles under cover with a free air circulation through the piles. Teak dries quickly and with little or no depreciation. [Anonymous. ,2005]

DURABILITY

Heartwoodteak:

The heart wood of teak is one of the most durability of woods. Graveyard test indicate the average life of teak 160 months(Madhya Pradesh). Heart wood has a moderate resistance to marine borers. Teak is almost immune to the attack of white ants and other insects, the immunity being due to the presence of toxic tectoquinones. Sapwood is perishable and quickly destroyed by rot, whiteants and borers. Sapwood is erratic to preservative treatment. Teak has

better resistance to decay against the whiterot fungi than the brownrot fungi. In older trees of inner heartwood is less resistant to decaying fungi than the outer heartwood, but in younger trees of entire heartwood has same resistance and comparable to the inner heartwood. Alcohol or benzene and methyl alcohol soluble heartwood is more durable. No marked difference between natural and plantation grown timber of teak but taken from different parts of a tree may differ in strength properties. Strength and specific gravity are higher at the bottom and top regions and lower in the intermediate segments. This variation is comparatively greater in plantation grown teak wood thanin a natural crop. Strength also increases with distance from the pith, but decreases near the periphery. Strength of young poles is low. [Anonymous. ,2005]

South Indian teak:

It has same properties of Madhya Pradesh teak(weight:95, beam strength:95, beam stiffness:85, suitability as a post:85, shock resisting ability:95, retention of shape:105, shear:115 and hardness:95). The acoustic content is very high but low equilibrium constant and penetration of water. South Indian teak tolerates changes in atmospheric humidity. It has good resistance to acids. It does not corrode iron, copper and aluminium. [Anonymous. ,2005]

PHYTOCHEMICAL STUDIES:

- Teak wood showed the value for cold water extraction(4. 00), hot water extraction(5. 68), petroleum ether extraction(3. 06), alcohol benzene(6. 59), pentosans(19. 43), lignin(30. 05), holocellulose(61. 11), α-cellulose(36. 31), hemi-cellulose A(11. 37), hemi cellulose B(2. 97), alcohol soluble portion of holo-cellulose(10. 46), acetyl content(1. 66), ash(1. 33) and

silica(0. 25%). The sugars present in the hydrolysate of cellulose fractions are xylose, glucose, mannose, galactose and arabinose. The wood waste contain carbohydrates and lignin. Starch, tannin or alkaloid has not been reported from the wood. Percentage of silica which is found in vitreous form in parenchyma. The wood also contains 4. 8% of caoutchouc in parenchyma of xylem. This imparts good abrasion resistance, resistance to mineral acids. $CaHPO_4$. $2H_2o$ has been reported in Japanese teak. [Anonymous. , 2005]

- In teak wood extractives, reported organic compounds are anthraquinones, naphthalene compounds and triterpenic and hemi-terpenic compounds. [Anonymous. , 2005]
- Teak wood contain reported chemical constituents are,
    - Squalene
    - Lapachol
    - Lapachonone
    - Deoxylapachol
    - Tectol
    - Dehydrotectol
    - 2-methyl anthraquinone(tectoquinone)
    - 2-hydroxy methyl anthraquinone
    - 3-methyl anthraquinone
    - 3-hydroxy 2-methyl anthraquinone
    - 4, 5 dihydroxy methyl anthraquinone

- 1-hydroxy 2-methyl anthraquinone
- 1, 4-dihydroxy 2- methyl anthraquinone
- Anthraquinone 2-carboxylic acid
- Anthraquinone 2- aldehyde
- 2-(3, 3-dimethyl allyl)1, 4-naphthaquuinone
- *O*-tolyl methyl ether
- Cis and trans 1, 4-polyisoprene
- 2-iodobenzoic acid
- Betulinic acid

- Teak wood also contains a number of free volatile fatty acids like formic ,acetic, propionic, butyric, crotonic and acrylic acids. [Anonymous. , 2005]

PHARMACOLOGICAL ACTIVITY

CYTOTOXIC ACTIVITY

- Methanolic extract of *T. grandis*wood showed 61%inhibition against chick embryo fibroblast cells. [Mahesh S Krishna and Jeyakumaran Nair A. 2010]

ANTI-OXIDANT ACTIVITY

- Anti-oxidant activity of *T. grandis* wood extracts(hexane, methanol, ethyl acetate, chloroform)were checked with DPPH and $ABTS^{+}$ free radical. Ethyl acetate wood extract showed very high inhibition activity(98. 6%)than the standard compounds. [Mahesh S Krishna and Jeyakumaran Nair A. , 2010]

ANTI- TERMITE ACTIVITY

- *T. grandis* wood of different ages and origins against the termite *C. Curvignathus* by anti-fedency test exhibited different degrees of termite mortality acetone, water extract showed highest mortality[Rudi *et al.* , 2012]

- Ethanolic extract of *T. grandis* heart wood (TGHV)showed significant anti-oxidant activity in DPPH(4-250µg/ml), nitric oxide and hydroxyl radical(250-400µm) in vitro assay methods. [Agnihotri A *et al. ,* 2013]

ACUTE TOXICITY

- Acute toxicity study (as per the OECD guidelines) showed no signs of toxicity upto a dose level of 2000mg/P. O ofethanolic extract. [Agnihorti A, Singh V. , 2013]

ANTI-HYPERGLYCEMIC ACTIVITY

- Ethanolic extract of (TGHW)heart wood of *T. grandis* showed significant anti-hyperglycemic effect($p<0.01$) in alloxan induced diabetic rats at the dose of 500 mg/kg bwpo. [AgnihortriA, Singh V. , 2013]

NEPHROPROTECTIVE ACTIVITY

- Ethanolic extract of (TGHW) heart wood of *T. grandis* showed significant nephroprotective effect in alloxan induced diabetic rats at the dose of 500 mg/kg bwp. o. [AgnihortriA, Singh V. , 2013]

ANTIMICROBIAL ACTIVITY

- Methanolic extract of *T. grandis*(root and aerial parts)showed inhibitory action against *Alternariacalani*and *Helminthosporium*(phytopathogenic fungi)[Srivastava SR. , 2013]

FLOWERS AND SEEDS

ETHNOMEDICAL INFORMATION

- Flowers are used for bronchitis, biliousness, urinary discharge[Sharma PV *et al. ,* 1986, KheraN*et al. ,* 2013]diuretic, depurative, anti-inflammatory, burningsensation, dipsia, leprosy, skindiseases, strangury, diabetes and vitiated conditions of pitta and kapha. Seed in poisoning, seed oil in skin disease. [Shiddamallayya N *et al. ,* 2008]

- Oil obtained from seeds and flowers promotes the growth of hair and is useful in eczema, ring worm and to check scabies. [Aradhana R *et al.* , 2010]
- Infusion of flowers is taken in congestion of liver. [Aradhana R *et al.* , 2010]

PHARMACOGNOSTICAL STUDIES

- T. grandis flowers shortly pedicellate, in large erect terminal branched tomentosecymose panicles 0. 3-0. 9m long with lanceolate bracts at the forks, bracts beneath the calyx 2. 5mm long, linear lanceolate. Calyx in flower 3mm long, stellatelytomentose, semiglobose-campanulate, the lobes 5 or 6 spreading, subequal 1. 2mm long.

PHYTOCHEMICAL STUDIES

- The preliminary phytochemical screening of the aqueous extract of flowers and seeds of *T. grandis* revealed the presence of phenolic compounds, carbohydrates, saponins, tanninsandflavonoids[Phalphale SG *et al.* , 2012]
- Methanolic extract of *T. grandis* seed showed the presence of flavonoids, tannins, glycosides, steroids. Petroleum ether extract of *T. grandis* seed contain carbohydrates, saponins, glycosides, flavonoids, tannins, steroids. This report is irrelavent components are denoted as present. [JangameCM, Burande MD. , 2013]

PHARMACOLOGICAL ACTIVITY

HAIR GROWTH ACTIVITY

- Hair growth initiation time was significantly reduced to half and greater number of hair follicles (64 and 51%) in anagenic phase by topical application of 5%, 10% petroleum seed extract of *T. grandis* than the standard 2%minoxidil (49% only)in albino mice was

observed. Hence this study provide support to the traditional claim of use of seeds as hair tonic. [Jaybhaye D *et al.* , 2010]

DIURECTIC ACTIVITY

- Oral administration of the aqueous extract of flowers and seeds of *T. grandis* in three doses (100, 200&400 mg/kg)showed diuretic activity in rats at 2, 4, 6, 8 and 24 hrs. Significant activity showed at different time interval and there was increase in urinary Na^+ and Cl^- excretion, when compared to hydrochlorthiazide. It was also reported that the LD_{50} value of aqueous extract. [Phalphale SG *et al.* , 2012]

ANTI-INFLAMMATORY ACTIVITY

- Methanolic and petroleum ether extract of *T. grandis* showed significant Þ>0. 04 and dose dependent (100, 200, 400mg/kgbwpo)anti-inflammatory activity in carrageenan induced foot pad oedema model. [JangameCM, Burande MD. , 2013]

ROOT

ETHNOMEDICAL INFORMATION

- Roots are useful in anuria and retention of urine, anaemia. [KheraN, Bhargava S. , 2013, Qudhia P][Jung M *et al.* , 2006]
- Traditionally roots are used in the treatment of anurea and urine retention. In the tribal community of Dhule District of Maharashtra is being using the root extract for their common diseases.

  [Pooja*et al*., 2010]

PHYTOCHEMICAL STUDIES

- A phytochemical tests showed that aqueous and water extracts of *T. grandis* rootspositive for carbohydrates, reducingsugars, alkaloids, glycosides, flavonoids, sterols and saponins. [Kavshik A *et al.* , 2009]
- The preliminary phytochemical study indicated the presence of flavonoids, and tannins. [Pooja*et al.* , 2010]

- *T. grandis* root contains lapachol, tectol, tectoquinone, β-sitosterol and a diterpene, tectogradinol. [Pooja*et al. ,* 2011]
- HPLC finger print of methanolic extract of *T. grandis* roots and aerial parts showed gallic acid 17. 48µg/ml, ferulic acid 7. 27µg/ml. [Srinivastava SR. , 2013]

PHARMACOLOGICAL ACTIVITY

ANTI- ULCER ACTIVITY

- Lapachol, a naphthaquinone isolated from the roots of *T. grandis* possesses anti-ulcerogenic effect when given at a dose of 5mg/kg/p. o twice daily for 3 days on subsequently induced experimental gastric and duodenal ulcers in rats and guinea pigs and this action appears to be associated with and effect on the protein content of the gastric juice and reversed aspirin induced changes in peptic activity protein and sialic acid. [Goel RK *et al. ,* 1987]

ANTI- INFLAMMATORY

- It was reported that both methanol and aqueous extract of *T. grandis* root significantly suppressed (P>0. 05) the development of paw oedemainduced by carrageenan in rats at the dose level of 500 mg /kg. [Kavshik A *et al. ,* 2009]

ANTI-OXIDANT ACTIVITY

- Maximum scavenging of nitric oxide, superoxide radicals the methanolic extract of *T. grandis* root was found to be 26. 61 and 46. 64% respectively at the concentration of 250µg/ml and compare with rutin as a standard. It indicates as its effective in scavenging free radicals and to be a powerful anti-oxidant. [Pooja*et al. ,* 2010]

ANTI-TUSSIVE ACTIVITY

- Aqueous and methanol extract of *T. grandis* roots significantly suppressed (P<0. 05) the asthmas in sulfur dioxide gas induced cough model in rats at the dose level of 500 mg/kg. [Kaushik A *etal. ,* 2011]

LD_{50}

- No toxic effect lethality of methanolic extract of *T. grandis* root was observed upto 3000 mg/kg p. o in rat only the consumption of food was increased by 20% the dose of 2000&3000 mg/kg during 4h but remaining normal afterwards. [Pooja*et al.* , 2011]

HYPOGLYCEMIC ACTIVIY

- Methanolic extract of *T. grandis* root exhibited significant ($p<0.05$) hypoglycaemicactivity inalloxan induced albino rats at 500mg/kg p. o dose level and compare with Glibenclamide. [Pooja*et al.* , 2011]

ANTI-PYRETIC ACTIVITY

- The methanolic root extract of *T. grandis* 250, 500mg showed significant ($p>0.5$) reduction in body temperature on yeast induced pyrexia in wistar albino rats and compared with paracetamol. It was claimed that the constituent lapachol may be responsible for the anti-pyretic activity. [Priyanka S*et al.* , 2011]

FRUITS

ETHNOMEDICAL INFORMATION

- Fruits are diuretic, demulcent, strangury, vesiclecalculi, pruritis, stomatitis[Aradhana R *et al.* , 2010]

PHARMACOGNOSTICAL STUDIES

- *T. grandis* fruits are hard, bony, irregularly globose, somewhat pointed at the apex, the pericarp soft with dense felted stellate hairs, 10-15 mm in diam, 4-celled, enveloped by light brown, bladder like calyx. [Kiritikar Basu. , 1987, Anonymous. , 2005]

PLANT MEDICINAL VALUE AND MALARIA CONTROL

Malaria, the disease, is a clinical diagnosis and traditional medicines have been used to treat symptomatic malaria for hundreds of years . These medicinal herbs are still used today by the majority of the rural populations in developing countries. A need for traditional medicine as a source of malaria treatment has been recognized in view of the difficulties faced in areas where populations are either unable to afford or access effective antimalarials or are unwilling to use allopathic medicines.

Natural products have played a key role in the discovery of leads for the development of drugs for malaria . For example, the bark of the Peruvian Cinchona tree was one of the anti-fever herbs that led to the discovery of natural quinine (an alkaloid), the first antimalarial drug which is still used today as well as several synthetic quinolones, particularly chloroquine This plant was used to treat malaria since the year 1632 . Another example includes artemisinin, which was isolated from a sweet or annual wormwood. Tree, *Artemisia annua*by Chinese researchers in 1972. This plant has been used as a traditional remedy for chills and fevers for more than 2000 years by the Chinese. Artemether, a derivative from artemisinin, is found to be more active than its precursor and is the most frequently used artemisinin derivative as a first line treatment for uncomplicated malaria (World Health Organization, 2008). New antimalarial leads are, therefore directed towards plant sources. There is a consensus that traditional medicines as antimalarials are effective but safety and efficacy concerns of these medicinal plants prevents them from being an integral part of the current health care systems. Plants, in general, constitute of a wide array of phytochemicals, and when used in high dosages the herbal remedies can elicit harmful effects on the body instead To assure the development of efficient and safe malaria phytomedicines, the uses of these

medicinal plants needs to be scientifically studied (i. e. critiqued and standardized). The pharmacological efficacy, phytochemical composition, as well as the toxicity of these plants needs to be investigated as potential antimalarial medicines.

DRUG DISCOVERY FROM ANTIMALARIAL NATURAL PRODUCTS

Drug discovery from natural products remain a focal point. Plants have been used for centuries for various purposes, including the treatment of diseases. The experimental use of plants has led to the discovery and isolation of drugs (i. e. the pharmaceutical active components within the plant) such as cocaine (*Erythroxylum coca*), opium (*Papaversomniferum*), and important antimalarial drugs such as artemisinin (*Artemisia annua*), quinine (*Cinchona* species) and their respective analogues. As a result, more emphasis is put on medicinal plants in the quest to develop novel drugs for malaria.

STATEMENT OF THE PROBLEM

Historically plants have been used as medicine to treat malaria. Today still, indigenous flora is used as curative means to treat disease and numerous ailments. This knowledge has been passed on orally from generation to generation among the locals. Even though these plants may be used as herbal remedies, in very few instances have their uses been recorded, and more specifically validated. As a result, this knowledge can become lost Although it is premature to conclude that herbal combinations can be used as effective antimalarials, the screening of herbal medicines provides a foundation for further exploration of their use as antimalarial medicines. There is a need to record and validate the medicinal uses of these plants to expand their use to include integration into modern medical healthcare systems. In this study an attempt was made to validate the ethno-medicinal uses of medicinal plants *Tectonagrandis*Linn. through botanical identification and biological assessment of its value as complementary medicine for malaria

CHAPTER-3

AIM AND OBJECTIVE

The resurgent idea of multicomponent drug therapy preferred to be better than monotherapy illustrated by the recent resolution of World Health Organization to support artemisinin based combination therapies(ACT) instead of mono therapy with artemisinin. Literature reveals that some flavonoid show anti-malarial activity(Dehydrosilibin, Di methyl allylcampferide)but rutin and silibin reported to be inactive chloroquine sensitive and resistant to *P.falciparum* were affected luteolin>quercetin>apigenin. When flavonoids were combined there was additive effect of the flavonoids. This indicates that flavonoid can play an important role highly endemic malaria region. Recent evidences showed common dietary flavonoids such as quercetin, apigenin, luteolin and kaempferol had both individual and synergetic effect against *P.falciparum* in-vitro. [Lehane A, Salibak .,2008,Ferreira JFS et al.,2010]

The urgent need for new anti-malarials due to massive worldwide use of chloroquine(CQ).The gold standard treatment of malaria was followed the reports of CQ resistant strains of *P.falciparum*. CQ resistant today has spread to the vast majority of malaria endemic areas rendering CQ increasingly ineffective. However inspite of prevalence of CQ resistance *P.falciparum* it continues to be widely used and this almost certainly contribute to the recent finding that malaria associated mortality is on the increase in Africa. Sulfodoxine-pyrimethamine (sp) a combination antifolate drug is the only other widely used inexpensive antimalarial but resistance is also leading to unacceptable levels of therapeutic failure in many areas in Asia, South America and Africa. The mid-term replacements are artemisinin

derivative which have very short half- lives and necessitates the use in combination with long acting drugs clearly additional new drugs are needed.

If we are to avoid an ever- increasing toll of malaria on tropical areas, it is imperative to rapidly put in to action strategic plans for the discovery and development of novel anti-malarial compound that are not encumbered by pre-existing mechanisms of drug resistance.[Fidock DA et al.,2004]

AIM:

To study the pharmacognostic , preliminary phytochemical, *invitro* antimalarial activity along with enhancement of *ex-vivo* Porcine skin wound healing model of the leaves of*Tectonagrandis*Linn. (Family:Verbenaceae).

OBJECTIVE:

The objective of the study was divided into three parts.

Part 1: **Phramacognostical study**:-

- Collection and authentication of plant
- Morphological study of the plant
- Microscopy of the leaf
    1. Anatomical study using light microscope
    2. SEM analysis
    3. Powder microscopy
    4. Microscopic schedules
- Physio-chemical parameters

1. Ash values
2. Loss on drying
3. Extractive values

Part 2: **Preliminary Phytochemical Screening**:-

- Preparation of ethyl acetate extract of *T.grandis*leaves (TGEAE)
- Qualitative analysis of the leaves for the presence of various phyto constituents
- Determination of flavonoid content, total phenolic content,
- determination trace elements by SEM
- Identification and quantitative determination of apigenin by HPTLC

Part 3: **Pharmacological study**:-.

- Acute toxicological study using brine shrimp lethality bioassay (BSLA)

  Production of *Artemia*nauplii

  Cytotoxicity bioassay

  Lethality concentration determination
- *In Vitro* anti malarial activity of TGEAE of the leaves
- In-vitro cultivation of *Plasmodium falciparum*

  Collection and identification of *Plasmodium falciparum* infected blood sample:

  Preparation of medium for cultivation of parasite

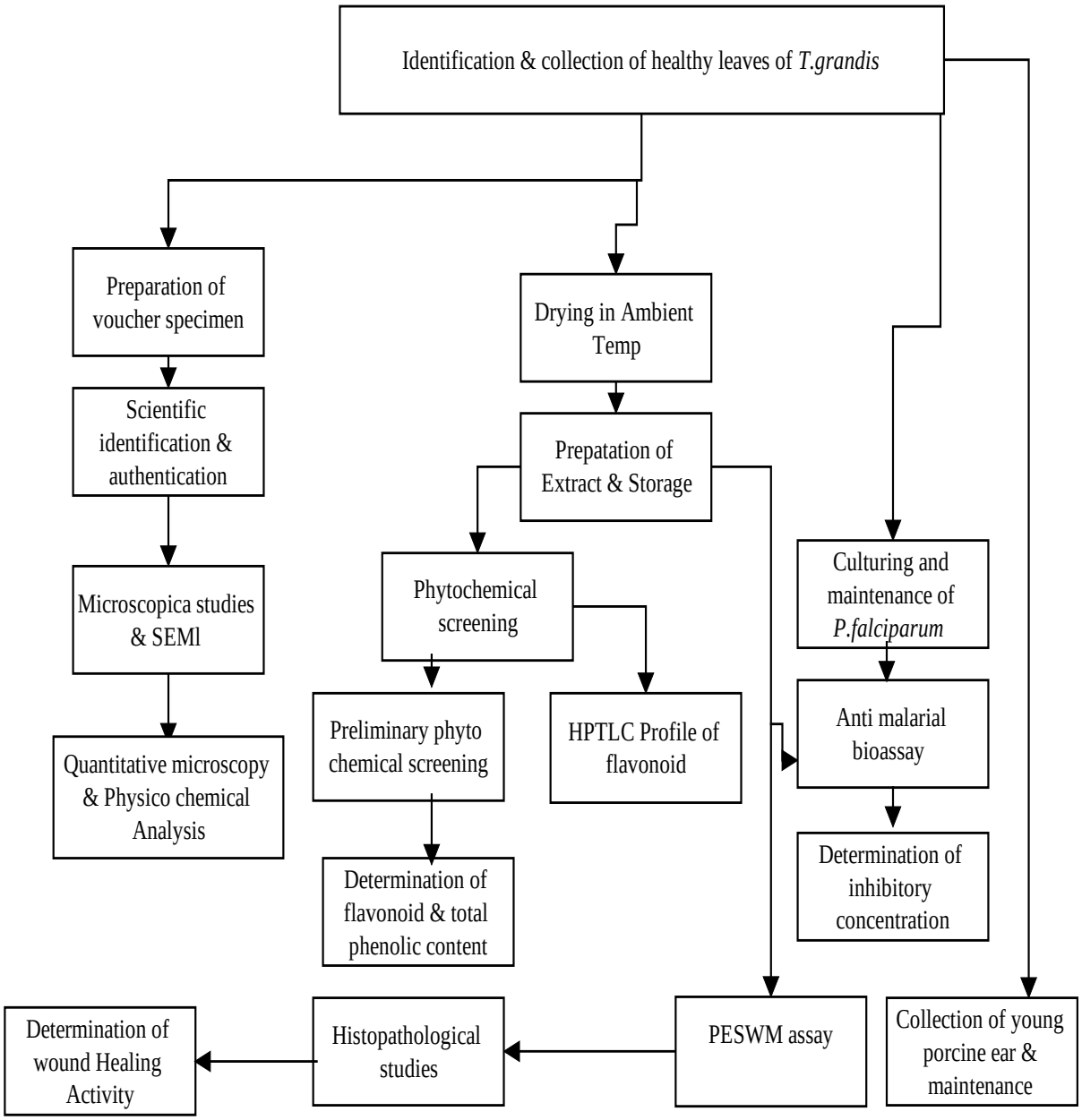
  In-vitro cultivation of *Plasmodium falciparum*

  Testing of Anti-malarial Sensitivity of TGEAE of the leaves
- To evaluate the effect of TGEAE of the leaves on the *ex-vivo* porcine skin wound healing model to provide scientific confirmation of the ethno medical claim.

CHAPTER – 4

MATERIALS AND METHODS

RESEARCH DESIGN



4.1. PLANT COLLECTION AND AUTHENTIFICATION

Leaves of the plant *Tectonagrandis*Linn. selected for our study was collected from **Karungal,Kanyakumari District**, Tamil Nadu, India during the month of July 2013 and was authenticated by**Dr.Stephen,** Department of Botany, American college, Madurai and **Dr.Sasikala** Director of Siddha Central Research Institute, Arunbakkam, Chennai.

Leaf drying and pulverizing

The leaves were collected and shade dried. It was powdered in a mixer. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

4.2. PHARMACOGNOSTIC STUDIES

Morphological and micro morphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies. Botanical identity of the plants is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many useful pharmacological active properties in the plant. If the botanical identity of the plant happens to be dubious or erratic, the entire work on the plant becomes invalid. Thus it is needless to stress the botanical identity of the crude drug is the threshold in the processes of pharmacological investigations. The researchers should be equipped with all possible diagnostic parameters of the plant on which the researchers plan to work.

4.2.1. Morphological studies of *Tectonagrandis*Linn.

Aerial part, leaf and petiole, flower and fruits were studied individually for its morphological characters by organoleptic test.

4.2.2. Microscopical studies on the leaf of *T.grandis*

Collection of specimen

Care was taken to select healthy plants and for normal organs. Leaf, Petiole specimens were collected from a healthy plant by making a cut with petioles.The materials were cut into pieces and immediately immersed in fixative fluid FAA (Formalin – 5ml + Acetic acid – 5ml +70% Ethyl alcohol – 90ml).

Dehydration

After 24 hours of fixing , the specimens were dehydrated with graded series of ethyl alcohol and tertiary-butyl alcohol as per the schedule given by Sass, 1940. The specimen is kept is in each grade of the fluid for about 6 hrs.Every time the fluid is decanted and immediately the specimen were flooded with next grade of fluid.

Infiltration with paraffin wax

After dehydration, the shavings of paraffin wax were added to the vial containing the plant material with pure TBA. The paraffin shavings are added every 30mts at about 40-45ºC four or five times. Then the vials were filled with wax without damaging the tissues. The vial filled with wax is kept open in warm condition to evaporate all TBA, leaving the specimen in pure molten wax. The specimen filled with pure molten wax for 2 or 3 times by decanting the old wax every time.

Casting to mold

A boat made out of chart board, by folding the margin, is used to prepare a mold of wax containing specimens. The paraffin along with the leaf and petiole specimen was poured into the boat. With the help of heated needles, the specimen were arranged in parallel rows with enough space in between the specimens. The block was then immersed in chilled water and allowed to cool for few hours.

Sectioning:

The paraffin embedded specimens were sectioned with the help of microtome**.** The thickness of the sections was 10-12μm. Dewaxing of the sections was by customary procedure. The sections were stained with **Toluidine blue** as per the method published by O'Brien *et al* (1964). Since toluidine blue is a poly chromatic strain, the straining results were remarkably good and some **cytochemical reactions** were also obtained. The dye rendered pink colour to the **cellulose** walls, blue to the lignified cells, dark green to **suberin**, violet to the **mucilage**, blue to the **protein** bodies etc. Where ever necessary sections were also stained with **safranin** and **fast-green** and potassium iodide (for starch).

For studying the stomatal morphology, venation patttern and trichome distribution, **paradermal sections** (sections taken parallel to the surface of leaf as well as **clearing** of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken.with Nikon labphot 2 Microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light were employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifigations of the figures are indicated by the scalebars. (Johansen DA, 1940, Purvis MJ *et.al*., 1966).

4.2.3. POWDER MICROSCOPY:

Maceration technique

Maceration is the process of separation of individual cells by selectively dissolving the pectic middle lamella between the cells. The middle lamella binds the cells with each other forming different tissues. The middle lamella is dissolved by employing a chemical that dissolves the lamella to free the cells to obtain their three dimensional view.

Maceration fluid

Jaffrey's maceration fluid is one that is commonly used for maceration (Johnsen DA, 1940). The fluid consists of equal volumes of 5% chromic acid and 5% nitric acid. The plant material is cut into small pieces and immersed in the maceration fluid. The fluid with the materials is kept at 55ºC for 3-5 hrs. Then the material is washed thoroughly with water and placed on a glass slide in a drop of safranin (0.5%) for 15-20 min. The stain is drained carefully and mounted with a drop of dilute glycerin. The cells are spread well with a needle and the material is covered with cover slip. The slide so prepared is examined under the microscope to study different components of the macerate.

4.2.4. MICROSCOPIC SCHEDULES (Wallis TE, 1953, Wallis TE, 1965, Iyengar MA, 1994, Anonymous, 2001)

The vein islet number, vein terminal number, stomatal number and stomatal index were determined on fresh leaves using standard procedures.

A. Vein islet number and Vein termination number

The term vein islet in used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of vein islets per sq.mm. Area is called vein islet number.

Vein terminal number may be defined as the number of vein terminals present in one sq., mm. area of the photosynthetic tissue.

B. Determination of Vein Islet Number and Vein Termination Number

Small square portion from the lamina region of the leaf was cleared in chloral hydrate, stained and mounted on a slide. A camera Lucida is set up and by means of a stage micrometer the paper is divided into squares of $1mm^2$ using a 16mm objective. The stage micrometer is then replaced by the cleared preparation and the veins are raced in four continuous squares, either in a square 2mm x 2mm (or) rectangle 1mm x 4 mm.

When counting, it is convenient to number each vein-islet on the tracing. Each numbered area must be completely enclosed by veins, and those which are incomplete are excluded from the count if cut by the top and left-hand sides of the square (or) rectangle but included if cut by the other two sides. Ten readings for vein islet and vein termination number were recorded.

C. Stomatal Index

It is the percentage, which the numbers of stomata from the total number of epidermal cells, each stoma being counted as one cell.

I, Stomatal index = S/S+E x 100

Where S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

D. Determination of Stomatal Index

The procedure adopted in the determinations of stomatal number was observed under high power (45 X).The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula.

4.2.5. MICROSCOPICAL STUDY OF LEAF USING SCANNING ELECTRON MICROSCOPE

Scanning Electron Microscope (SEM)

Movement of beam of foccussed electrons across an object forms a 3D image on a cathode - ray tube in a Scanning Eectron Microscope and it reads both the electrons scattered by the object and the secondary electrons produced by it. The electromagnetic lenses are used in SEM and focussing is done by the current. On photographic plate of screen the image is projected which gives comprehensive, quasi 3-D representation of the objects gives the ultra structure of plant cells. In addition , shows the unsuspected details and any undescribed characters. In other words the micrograph from SEM, shows the best possible structural details of the specimens. (Robards, 1970)

Usage

SEM info was handled as conventional character (or) character complexes as “pure” information without being broken down (or) interpreted as individual character using computer processing. The SEM information can be used some what at the superficial level just described to assist in solving taxonomic problem by confirming, changing (or) other grounds.

It is also used often as diagnostic feature to avoid misleading by over simplified descriptions and one may find new kinds of microstructures not previously recognised and apprently simple structures may be extremely complex. Remarkably, poor conventional descriptions enabling taxonomic process of reducing a complex pattern to a few simple characters (Heywood VH, 1971). SEM plays a vital role when a specimen need to be satisfactorily defined in terms of characters. For most biological materials, maximum information is obtained by employing light and electron microscopy jointly and an attempt was made by applying SEM to the leaf of *T.grandis,* to pinpoint the positions of specific characters with in the cell, which can be easily seen in final image.

SEM sample preparation

Sample for SEM analysis were mounted on the specimen stub using carbon adhesive sheet. Small sample were mounted with I sq. cm glass slide And kept in carbon adhesive sheet.. Samples were coated with gold to a thickness of 100 AO using hitachi vacuum evaporator. Coated sample were analysed in a Hitachi Scanning electron Microscope 3000 H model.

4.2.6. PHYSICOCHEMICAL PARAMETERS: (Anonymous, 1996, 1998, 2001)

Determination of Ash Values

Ash Value

The ash values were determined by using air dried powder of the leaf as per the official method.

Total ash

Two grams of the air dried leaf powder was accurately weighed in a silica crucible separately. The powder was scattered into a fine even layer on the bottom of the crucible and incinerated by gradually increasing the temperature not exceeding 450^{o}c, until free from

carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried powder was calculated.

Water soluble Ash

The ash obtained from the total ash procedure was boiled with 25ml of water for 5 minutes and the insoluble matter was collected on an ash less filter paper and washed with hot water. Then it was ignited for 15 minutes at a temperature not exceeding 450^{o}c. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried powder.

Acid insoluble ash

The ash obtained from the total ash was boiled for five minutes with 25ml of dilute hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, dried and weighed. The percentage of acid insoluble ash with reference to the air dried drug was calculated.

Determination of Loss on Drying

For the determination of loss on drying, the method described by Wallis was followed. One gram of dried powdered leaf was accurately weighed in a tarred Petri dish, previously dried under the conditions specified in IP'96. The powder was distributed as evenly as practicable, by gentle sidewise shaking. The dish was dried in an oven at 100 – 105°C for 1 hour. It was cooled in desiccators and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

Extractive Values (Individual Solvent)

❁Petroleum Ether Soluble Extractive Value

Five gram of the coarsely powder was macerated separately with 100ml of petroleum ether in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of petroleum ether. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the petroleum ether soluble extractive value was calculated with reference to the air dried powder.

❁Ethyl Acetate Soluble Extractive Value

Five gram of the coarsely powder was macerated separately with 100ml of ethyl acetate in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethyl acetate. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the ethyl acetate soluble extractive value was calculated with reference to the air dried powder.

❁Ethanol Soluble Extractive Value

Five gram of the coarsely powder was macerated separately with 100ml of ethanol in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethanol. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the ethanol soluble extractive value was calculated with reference to the air dried powder.

❁Water Soluble Extractive Value

Five gram of the coarsely powder was macerated separately with 100ml of chloroform water in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of chloroform water. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the chloroform water soluble extractive value was calculated with reference to the air dried powder.

4.3. PHYTOCHEMICAL STUDIES

[Anoymous, 1998, Chaudhri RD, 1999, Kokate CK, 2005, Agarwal, 2007, Horbone JB, 1973]

4.3.1 PRELIMINARY PHYTOCHEMICAL SCREENING

PREPARATION OF EXTRACT

The leaf powder was sieved in a No.60 sieve and refluxed with ethyl acetate for 4hrs and filtered. The filtrate evaporated under vacuum (Rotavapor RII, Buchi). The pale green residue obtained (TGEAE) was stored in the refrigerator until further use.

TEST FOR ALKALOIDS

Various procedures to liberate alkaloids

- Powdered drug was mixed thoroughly with 1ml of 10% ammonia solution and then extracted for 10 minutes with 5 ml methanol, under reflux. The filtrate was then concentrated.
- Powdered drug was mixed thoroughly with 1ml of 10% sodium carbonate solution and then extracted for 10 minutes with 5ml methanol, under reflux. The filtrate was then concentrated.

- Powdered drug was ground in a mortar for about 1 minute with 2ml of 10% ammonia solution and then thoroughly mixed with 7 gram basic Aluminium oxide. The mixture was then loosely packed into a glass column and 10ml chloroform was added, eluted, dried and methanol was added.
- Powdered drug was shaken for 15 minutes with 15ml of 0.1 N sulphuric acid and then filtered. The filter was washed with 0.1 N sulphuric acid to a volume of 20ml filtrate; 1ml concentrated ammonia was then added. The mixture was then shaken with two portions of 10ml diethyl ether. The ether was dried over anhydrous sodium sulphate, filtered and evaporated to dryness and the resulting residue was dissolved in methanol.
- Powdered drug was mixed with one gram of calcium hydroxide and 5ml of water, made into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. 20ml of 90% alcohol was added, mixed well and then refluxed for half an hour on a water bath. It was then filtered and the alcohol was evaporated. To that dilute sulphuric acid was added.

The above made extracts were tested with various alkaloid reagents as follows.

1. Mayer's reagent

2. Dragendorff's reagent

3. Hager's reagent

4. Wagner's reagent

Test for purine group (Murexide test)

The residue obtained after the evaporation of chloroform was treated with 1ml of hydrochloric acid in a porcelain dish and 0.1g of potassium chlorate was added and evaporated

to dryness on water bath. Then the residue was exposed to the vapors of dilute ammonia solution.

TEST FOR CARBOHYDRATES

Molisch's test

- The aqueous extract of the powdered material was treated with alcoholic solution of α - naphthol in the presence of sulphuric acid**.**

Fehling's test

- The aqueous extract of the powdered material was treated with Fehling's I and II solution and heated on a boiling water bath.

Benedict's test

- The aqueous extract of the powdered drug was treated with Benedict's reagent and heated over a water bath.

TEST FOR GLYCOSIDES

General test

- **Test A**

200 mg of the powdered drug was extracted with 5ml of dilute sulphuric acid by warming on a water bath, filtered and neutralized with 5% sodium hydroxide solution. Then 0.1ml of Fehling's solution I and II were added, until it becomes alkaline and heated on a water bath for 2 minutes.

- **Test B**

200 mg of the powdered drug was extracted with 5ml of water instead of sulphuric acid. Boiled and equal amount of water was added instead of sodium hydroxide solution.

Then 0.1ml of Fehling's solution I and II were added, until it becomes alkaline and heated on a water bath for 2 minutes.

Anthraquinones

- **Borntrager's test**

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The inorganic layer was separated and ammonia solution was added slowly.

- **Modified Borntrager's test**

About 0.1gram of the powdered leaf was boiled for two minutes with dilute hydrochloric acid and few drops of ferric chloride solution was added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well.

Test for cyanogenetic glycosides

Small quantity of the powdered leaf was placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place.

Test for cardiac glycosides

- **Keller Killiani test**

About 1gram of the powdered leaf was boiled with 10ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3ml

of glacial acetic acid containing a trace of ferric chloride. To this 3ml of concentrated sulphuric acid was added along the sides of the test tube carefully.

- **Raymond Test**

  To the alcoholic extract of the leaf, hot methanolic alkali was added.

- **Legal's Test**

  To the alcoholic extract of the powdered drug, pyridine and alkaline sodium nitro pruside solution were added.

Coumarin glycosides

A small amount of powdered leaf was placed in test tube and covered with a filter paper moistened with dilute sodium hydroxide solution. The covered test tube was placed on water bath for several minutes. Then the paper was removed and exposed to UV light.

TEST FOR PHYTOSTEROLS

The powdered leaf was first extracted with petroleum ether and evaporated. The residue obtained was dissolved in chloroform and tested for sterols.

- **Salkowski Test**

Few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside.

- **Libermann – Burchard's Test**

To the chloroform solution few drops of acetic anhydride was added and mixed well. 1ml of concentrated sulphuric acid was added through the sides of the test tube and set aside for a while.

Test for Saponins

About 0.5gram of the powdered leaf was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5ml of the filtrate was then diluted with water and shaken vigorously**.**

Determination of Foaming Index

One gram of the coarsely powdered leaf was weighed and transferred to 500 ml conical flask containing 100 ml of boiling water. The flask was maintained at moderate boiling, at 80-90^{o}C for about 30 minutes. Then it was cooled and filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100 ml (V_1).

Ten Stoppard test tubes were cleaned (height 16 cm, diameter 1.6 cm) and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3ml up to 10ml and adjusted the volume of the liquid in each tube with water to 10ml. Then the tubes were Stoppard and shaken lengthwise for 15 seconds, uniformly and allowed to stand for 15 minutes and measured the length of the foam in every tube.

TEST FOR TANNINS

To the aqueous extract of the powdered leaf, few drops of ferric chloride solution were added.

- **Gold beater's skin test**

2% hydrochloric acid was added to a small piece of gold beater skin and rinsed with distilled water and placed in the solution to be tested for five minutes. Then washed with distilled water and transferred to a 1% ferrous sulphate solution.

TEST FOR PROTEINS AND FREE AMINOACIDS

- **Millon's test**

The acidulous alcoholic extract of the powdered leaf was heated with Millon's reagent.

- **Biuret test**

To the alcoholic extract of the powdered leaf 1ml of dilute sodium hydroxide was added. Followed by this one drop of very dilute copper sulphate solution was added.

- **Ninhydrin Test**

To the extract of the powdered drug, ninhydrin solution was added, and boiled.

TEST FOR MUCILAGE

To the aqueous extract of the powdered leaf, ruthenium red solution was added.

TEST FOR FLAVONOIDS

- **Shinoda Test**

A little amount of the powdered leaf was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added, and boiled for 5 minutes.

- **Alkaline reagent test**

To the alcoholic extract of the powdered leaf, few drop of sodium hydroxide solution was added.

- **Zinc Hydrochloride Test**

To the alcoholic extract, mixture of zinc dust and concentrated hydrochloric acid was added.

TEST FOR TERPENOIDS

The powdered leaf was shaken with petroleum ether and filtered. The filtrate was evaporated and the residue was dissolved in small amount of chloroform. To the chloroform solution tin and thionyl chloride were added.

TEST FOR VOLATILE OIL

About 100gram of fresh leaves, were taken in a volatile oil Clevenger apparatus and subjected to hydro distillation for four hours.

TEST FOR FIXED OIL

A small amount of the powdered leaf was pressed in between in the filter paper and the paper was heated in an oven at 105^{o}C for 10 minutes.

4.3.2. FLUORESCENCE ANALYSIS

Powdered leaf material of*T.grandis* was subjected to analysis under UV light after treatment with various chemical and organic reagents like Ethanol, Ethyl acetate, Chloroform, Water, 50% sulphuric acid, 10% sodium hydroxide, 50% nitric acid and dried leaf powder (Horbone JB, 1973).

4.3.3. ESTIMATION OF FLAVONOID CONTENT

[Chang CC *et al*.,2002, Mabry TG *et al*., 1970 and Siddiquie MA *et al.,* 2010].

The flavonoid content of plant extract was estimated by aluminium chloride method. In this method, aluminium chloride complexes with flavonoids of C3-C5 hydroxyl group and to produce intense colour in acidic medium. The intensity of the colour is proportional to the amount of flavonoids and can be estimated as quercetin equivalent at wavelength of 415nm.

Materials required

1. Ethyl acetate extract of leaves of *Tectonagrandis*(TGEAE)
2. 10%w/v aluminium chloride
3. 1M Potassium acetate
4. 95%v/v ethanol

Procedure

0.5ml of TGEAE (1mg/ml) was transferred to a test tube. To this solution, 0.1ml of aluminium chloride, 0.1ml of potassium acetate, 1.5ml ethanol and made to 5ml with distilled water. The mixture was allowed to stand for 30 min with intermittent shaking. The absorbance was measured at 415nm. The calibration curve was generated using quercetin as a standard at different concentrations (5-50µg/ml). The reaction mixture without aluminium chloride was used as a blank. The flavonoids content in TGEAE was expressed as mg of quercetin equivalent per gram of extract.

4.3.4. ESTIMATION OF TOTAL PHENOLIC CONTENT (Singleton VL *et al.*, 1979,Gouthamchandra K *et al.*, 2010)

Principle

The total phenolic content of the TGEAE was determined by FolinCio-calteau reagent. This reagent consists of phosphotungstate and phosphomolybdate mixture which is reduced to mixture of blue molybdenum and tungsten oxides while phenolic content of the extract was oxidized. The intensity of colour is proportional to the amount of phenolic content of the extract and which was measured at 765nm. The total phenolic content in TGEAE was expressed as milligrams of gallic acid equivalent (GAE) per gm of extract.

Materials

1. Ethyl acetate extract of leaves of *Tectonagrandis*(TGEAE)
2. 10%w/v sodium carbonate solution
3. Gallic acid
4. Folin-Ciocalcateau reagent

Procedure

0.5ml and 1ml of TGEAE was transferred into separate test tube. To this solution, FCR 0.5ml and 1ml of sodium carbonate were added and final volume made upto 10ml with distilled water. The mixture was allowed to stand for 1hr with intermittent shaking. The absorbance was measured at 765nm. A calibration curve was generated using gallic acid as a standard at different concentrations (2, 4, 6, 8, 10µg/ml). The reaction mixture without sample was used as a blank. The total phenolic content in TGEAE was expressed as milligrams of gallic acid equivalent (GAE) per g of extract.

4.3.5 DETERMINATION OF TRACE ELEMENTS IN LEAF OF *T.grandis* BY ENERGY DISPERSIVE X-RAY SPECTROMETER (EDS)

The SEM allows the observation of materials in macro and submicron ranges. SEM is capable of generating 3-D images for analysis of topographic features . When SEM is used along with EDS the analyst can perform an elemental analysis on specimens of microscopic sections or contaminants that may be present.

EDS analytical capabilities

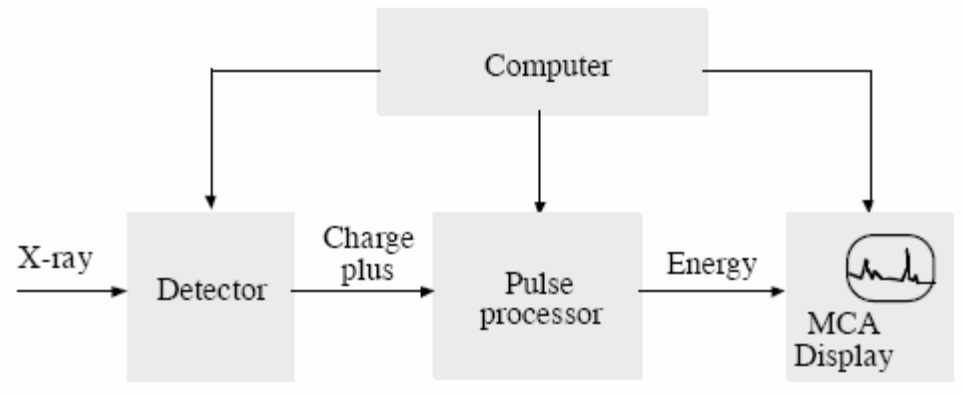
Backscattered electron images in the SEM display compositional contrast that results from different atomic number elements and their distribution. EDS is used to find particular elements are and their Atomic % . The Y-axis shows the counts (number of X-rays received

and processed by the detector) and the X-axis shows the energy level of those counts[Bob Hofner].

By Viewing 3-D images of specimens solves some of the problem in an analysis and it is also necessary to detect different elements associated with the specimen. This is accomplished by using the “built-in” spectrometer called an Energy Dispersive X-ray Spectrometer.

EDS system comprises of 3 basic components

1. An X-ray Detector - detects and converts X-ray into electronic signals.
2. A Pulse Processor - measures the electronic signals to find out energy of each X-ray detected; and
3. A Multiple Channel Analyser - interprets and displays analytical data.



EDS is an analytical technique in which the specimen emits X-rays due to the bombardment of electron beam on it which is used to identify the elemental composition of the specimen due to the ejection of electrons from the atoms on the specimen surface. To explain further, when the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the specimens surface. A resulting electron vacancy is filled by an electron from a higher shell, and an X-ray is emitted to balance the energy difference between the two

electrons. The EDS X-ray detector measures the number of emitted X-rays emitted versus their energy.

The energy of the X-ray is characteristic of the element from which the X-ray was emitted. A spectrum of the energy versus relative counts of the detected x-rays is obtained and evaluated for the determinations of the elements.

4.3.6HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY OF TGEAE:

High performance thin layer chromatography (HPTLC) is a modern adaptation of TLC with improved versatility, separation efficiency and detection limits. HPTLC is a useful tool for identification of plant extract because each plant species produces a distinct chromatogram, with unique marker compounds used for the plant identification. It is used as a quality control tool since comparison of chromatograms of different lots can demonstrate the similarities and differences between the test samples and their standard chemical markers. HPTLC is a reliable method for quantification of nanogram level even when present in complex formation. HPTLC fingerprint analysis is used for rapid identity check, for monitoring purity of drugs, for detection of adulterants, for determining whether a material is derived from a defined botanical species and also to know whether the constituents are clearly characterized

(Wagner H *et al.,* 1996)

4.4 PHARMACOLOGICAL STUDIES:

4.4.1 ACUTE TOXICOLOGICAL STUDY USING BRINE SHRIMP LETHALITY ASSAY (BSLA)

(Michael A.S *et.al.*, 1956, Vanhaecke P *et.al.*,1981, Sleet R.B and Brendel K.,1983)

The importance of medicinal plants and traditional health systems solving the health care problems of the world is gaining increasing attention. Most of the developing countries have adopted traditional medical practice as an integral part of their culture.

In order to study the toxicity of these medicinal plants we performed **Brine Shrimp Lethality Bioassay** which based on the ability to kill laboratory cultured brine shrimp (***Artemianauplii***). The brine shrimp assay is a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials.

The method is attractive because it is very simple, inexpensive and low toxin amount are sufficient to perform the test in the micro well scale.

Production of *Artemia*nauplii

*Artemia*is a non-selective filter feeder of organic detritus, micro algae and bacteria. Artemia are naturally found in saltpans, hyper saline lakes and coastal lagoons as well as in the man made saltpans. When the cysts are inoculated in seawater for 24 hrs, the free-swimming nauplii are hatched out.

Cytotoxicity Bioassay

Ten nauplli were drawn through a glass capillary and placed in each vial containing 4.5ml of brine solution. In each experiment, 0.5ml of the extract was added to 4.5 ml of brine solution and maintained at room temperature for 24 hrs under the light and surviving larvae

were counted. Experiment was conducted along with control (vehicle treated), different concentrations of the aqueous extract (100-7500 ppm) & volatile oil (5-50 ppm) in a set of three tubes per dose.

Lethality Concentration Determination

The percentage lethality was determined by comparing the mean surviving larval of the test and control tubes. LC_{50} value was obtained from the best – fit line, plotted concentration verses percentage lethality. Podophyllotoxin was used as a positive control in the bio assay.

Abbot's formula :

$$\textbf{Corrected mortality (\%)} = \frac{\textbf{Test mortality (\%) - Control mortality (\%)}}{\textbf{100 - Control mortality (\%)}} \times \textbf{100}$$

4.4.2 *In Vitro* ANTI MALARIAL ACTIVITY OF TGEAE THE OF LEAVES

INTRODUCTION[WHO Geneva.,1998]

Disease caused by protozoa are responsible for considerable mortality and morbidity,especially in the developing world.Malaria is by far the world's most important tropical parasitic disease,than any other communicable disease except tuberculosis.

It is a public health problem,inhabited by a total of some 2400 million people 40% of the world's population.Around 3 million people were affected by malaria in Indian subcontinent.

The causative agents in humans are four species of *plasmodium protozoa,P.falciparum,P.vivax,P.ovale and P.malariae of these P.falciparum* accounts for the majority of infections and is the most lethal.

In 1950s,itt was confidently expected that the disease would be eradicated because of effective drugs which would eliminate *P.falciparum* infection in man and potent insecticides which would destroy the Anopheles vector mosquitoes.Despite the efforts,today,malaria has

again reacted epidemic proportions on a world wide scale due to resistance of *P.falciparum* to clinically useful drugs such as chloroquine and pyrimethamine.Resistance of the vector mosquitoes to DDT & their insecticides has also contributed to the resurgence of malaria.

Many plant species are used in the preparation of traditional medicines for the treatment of protozoal diseases and plants are the sources of the clinically used antiprotozoal drugs quinine,artemisinin(from *Artemisia annua*).Now inexpensive and affordable new drug is needed.

The ethno medical information of *T.grandis* reveals that it has been used for the treatment of malaria .The aqueous,hydro methanol and chloroform extract of leaves,roots and stem barks of *P.inermis* induced a significant decrease of parasite proliferation when evaluated for antimalarial activity[Traore Keita F et al .,2000] .The present study focused on evaluation of the antimalarial activity of the ethyl acetate extract of *T.grandis*against*P.falciparum*.

In-vitro* cultivation of *Plasmodium falciparum[Usha Devi C and Pillai CR]

Collection and identification of *Plasmodium falciparum* infected blood sample:

Plasmodium falciparum infected blood was collected and identified by P.Udayakumar, District Malarial Officer, Ramnad, Tamilnadu from Primary Health Centre, Pamban after getting the necessary consent from the patient.

Preparation of medium for cultivation of parasite

10.4 gms of RPMI-1640 was dissolved in 900ml of double distilled water and 5.94 gms of HEPES was added to this solution for buffering.The volume was madeup to 960 ml and this solution was sterilized by filtration through a Millipore filter of 0.22µm porosity.This filtered solution was divided into aliquots and canbe stored at 4˚C up to a period of one month.The pH was adjusted to7.4 by the addition of 4.2 ml of sterile 5%Sodium bicarbonate.This incomplete

medium was used for washing the erythrocytes.Complete media was prepared by the addition of 10 ml of normal human serum to this incomplete medium.

Serum

Blood was collected without anticoagulant and kept overnight at 4˚C.The serum was separated by centrifugation.The type of serum depends on the cells to be used.The best suited is AB^+serum because this is compatible with any type of human cells.The serum can be stored frozen at -20˚C.

Erythrocytes

The cells were obtained from the blood collected in ACD(Acid Citrate Dextrose).The cells can be stored at 4˚C for one month.Any type of cells (erythrocyte)can be used with AB^+ serum,butA^+cell are preferable.

Red cells are prepared as follows

Appropriate volume of blood was taken in a centrifuge tube and centrifuged for 10 minutes at 650g(2000rpm).The supernatant portion along with the buffy coat was removed.The cells were mixed and resuspended in the incomplete medium and centrifuged.This washing procedure was repeated thrice.Finally the supernatant was removed and the cells resuspended in equal volume of complete medium to make a 50%suspension.

Method

The infected blood was collected from a malaria patient.The blood was processed(washed)exactly like the washing procedure followed for the normal erythrocytes and finally a 50%suspension was prepared.An appropriate volumeof the infected cells,was added to the uninfected erythrocytes to get an initial parasitaemia of 0.1%.This suspension was diluted with complete medium to make an 8% cell suspension and it was dispensed in the

culture vials.The vial should contain a thin layer of erythrocytes and the height of the medium should not exceed 4mm.

The vials were placed in a desiccator with a candle.The candle was lit and the cover put on with the stopcock open.When the candle flame goes out,the stopcock was closed and the desiccator was set in an incubator at 37˚C.

The medium of the culture should be changed after every 24 hours and fresh medium added.Slides were prepared to see the parasitaemia.Then the subculture of the cells were done.

The percentage of infected cells was calculated by examining under the oil immersion lens(1000 x).The number of infected RBC'$_S$and number of non infected RBC$_S$ were counted to estimate the percentage of the parasite for 100 RBCs.

Testing of Anti-malarial Sensitivity of TGEAE of the leaves

Micro *in-vitro* test against the ethanol extract of the plant was carried out in microtitre plates.(Pl250µl of infected blood from *in-vitro*cultivation of *P.falciparum* was taken in five wells each.The first well was kept as control.In the second well 50µl of Chloroquine at the concentration of 0.01mg/µl was added and kept as a standard.

In the 3rd,4th and 5th wells 50µl of ethanol extract of *T.grandis* in the concentration 0.01mg/50µl was added and incubated at37˚C in an incubator under an atmosphere with 7% CO_2 and low oxygen .Slides were prepared during '0'hrs and incubation of 48 hrs,72 hrs and 96 hrs from all the wells(control,standard,test)and stained with JSB I and IIexamined under oil immersion lens and the percentage of infected cells was calculated as explained.

4.4.3 EFFECT OF TGEAE LEAVES ON *ex-vivo* PORCINE SKIN WOUND HEALING MODEL

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury an inflammatory response occurs and the cells below thedermis begins to increased collagen production. Later the epithelial tissue is regenerated.

The objective of screening of drugs for healing activity based on various measures on healing (Nayak S 2006).

Wound healing evaluated by *ex-vivo* porcine skin wound healing model (PSWHM). Architecture of pig skin is closer to human (Bollen Peter JA *et al*, 1999, Laber*et al*, 2002). Porcine model is an excellent tool for the evaluation of therapeutic agent meant for wound healing (Sullivan TP *et al*, 2001).

Porcine (6 months old) ears were obtained from a local slaughter house were washed and disinfected with PBS and 70% ethanol respectively. Circular porcine skin (6mm diameter) taken by using circular biopsy punch. Subsequently on the excised skin small circular wound (3mm diameter) made by using circular biopsy punch. Removing epidermis and upper dermis from the centre for making the wound under sterile conditions. The PSWHMs were divided into five groups (n=6). Control, **1%, 2%, 3%** test drug (TGEAE) Ointment, Standard drug (Mupirocin) 2% Ointment applied on the wound and immersed in PBS. Incubate the PSWHM with 5% CO_2 at 37°C for 2 days. Histopathological evaluation done after staining with hematoxylin / Eosin. The migration was normalized with the PBS group and expressed as mean % ± SE. statistical analysis was performed using one way analysis of variance (ANOVA). P value 0.01 was considered to be statistically significant (Khamule R *et al*, 2012).

CHAPTER - 5

RESULTS

5.1 PHARMACOGNOSY: (Kiritikar basu 1987, Anonymous 2002,2005)

5.1.1 Morphological features of *T.grandis* Linn.

It is a large deciduous tree, 10-20m tall(Plate-1, Fig -1,1A).

Leaves : (Plate -2,3, Fig 1, 1A)

Shape : Elliptic or obovate,acute or acuminate,the upper surfacerough But usually glabrous, the lower clothed with dense stellategrey or tawny tomentum, petiolate, opposite or whorled, large, entire

Size : Length 30-60cm × 20-30 cm.

Colour : Green

Margin : Entire or crenulate

Base : Usuallycuneate: main nerves 8-10 pairs with 2 or 3 Large branches near the edge of the leaf, joined by numerous Parallel transverse veins

Flowers :(Plate-4,5Fig 1,1A)

Shape :Shortly pedicellate,in large erect terminal branched tomentose Cymose panicles 0.3-0.9 m long,with lanceolate bracts at the forks. : Bracts beneath the calyx2.5mm.long,linear lanceolate

Size :Large erect

Colour :White

Taste :Sweet-scented

FIG - 1

Diagram showing the Aerial Parts of ***T.grandis***

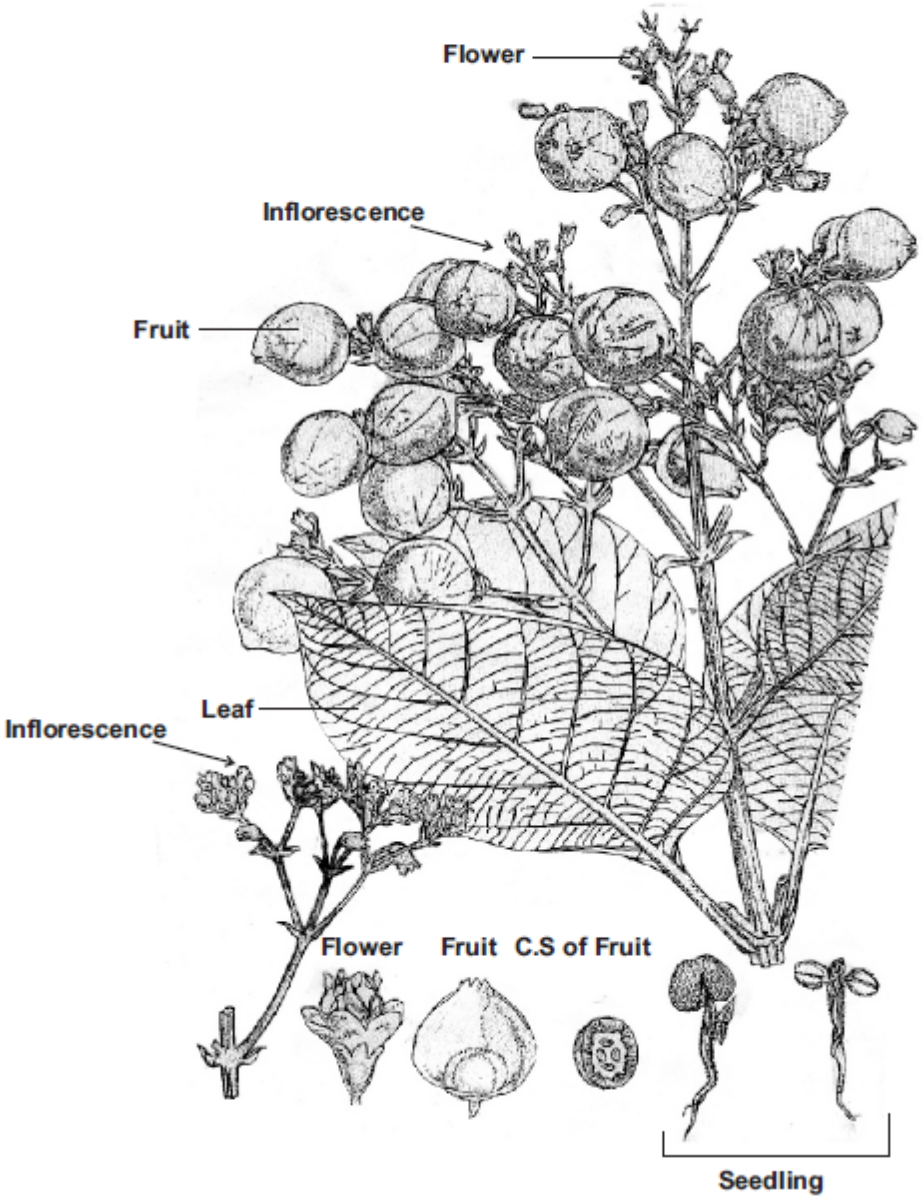


FIG - 1A

T.grandis Aerial Parts

Plate 1

Habit and Habitat of *T.grandis*

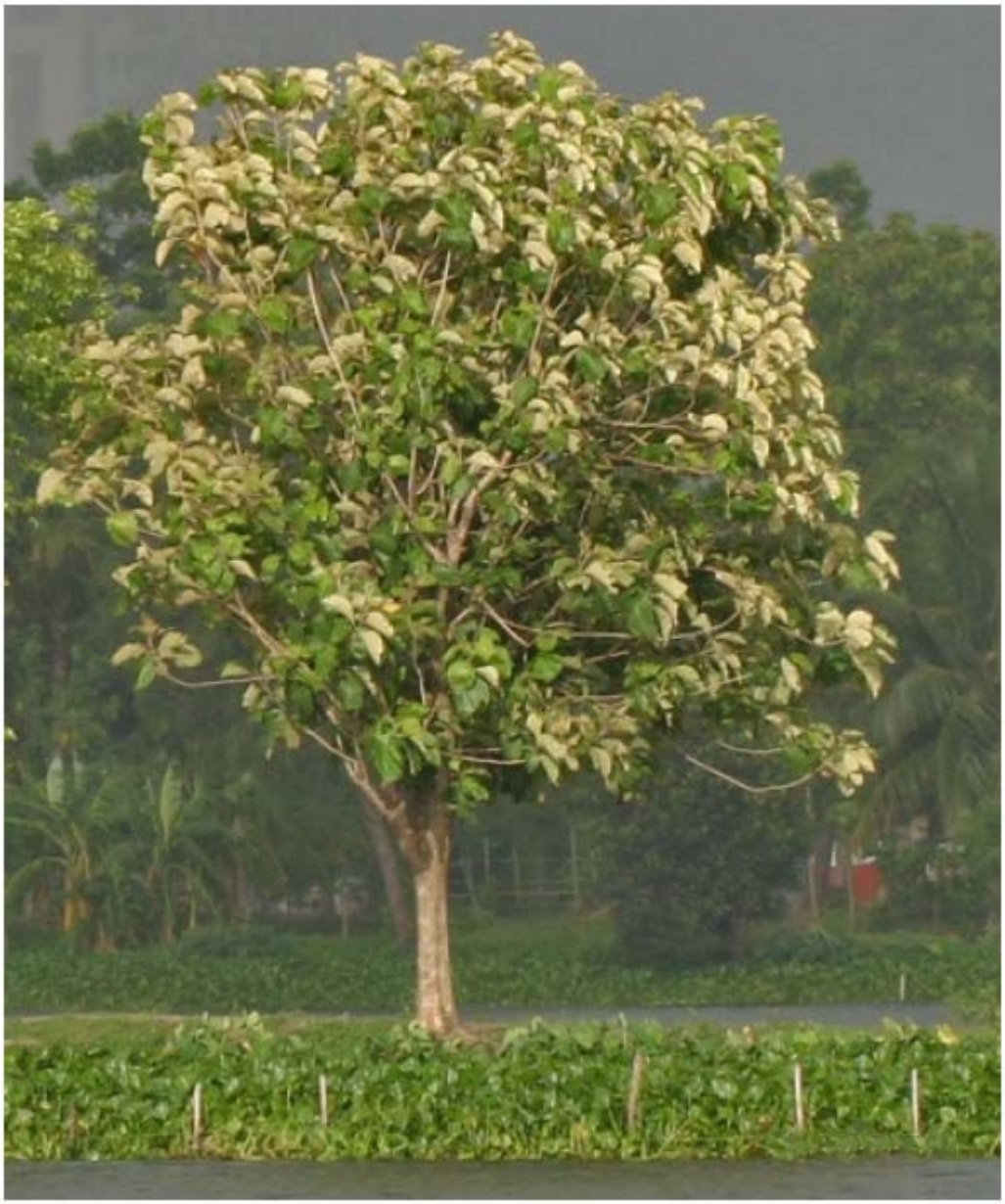


Plate 2

Leaf Arrangement of *T.grandis*

Plate 3

Dorsal and Ventral view of the Leaves *T.grandis*

Plate 4

Inflorescence of *T.grandis*

Plate 5

Flowers of *T.grandis*

Plate 6

Fruits of *T.grandis*

Plate 7

Seeds of *T.grandis*

Calyx :stellately tomentose semi globose campanulate,3mm long,5-6 lobed,enlarged and becomingbladder like infruit,ovoid or urceolate

Corolla :White,tube short,limb with 5-6short subequal spreading Imbricate lobes

Stamens :Inserted near the base of the corolla,equal,exserted(5-6)

Anthers :ovate or oblong with distinct parallel cells.

Ovary :Fleshy,4-celled,ovule solitary in each cell,style linear,stigma shortly 2-fid.

Fruits:(Plate-6, Fig 1, 1A)

Shape : DrupeHard,bony,irregularly globose,somewhat pointed at the apex enclosed in the enlarged calyx

Size :10-15mm in diameter

Colour :Stellate hairy,enveloped by light brown

Endocarp :Thick, bony,4-celled

Seeds:(Plate-7)

Seeds 1-3,rarely 4 in a fruit,marble-white,ovate,4-8mm long.

5.1.2 MICROSCOPY OF THE LEAF:

The leaf is dorsiventral with prominent midrib. Lamina is thick dorsiventral differentiated.

Plate 8

T.S of Leaf of *T. grandis* through the midrib

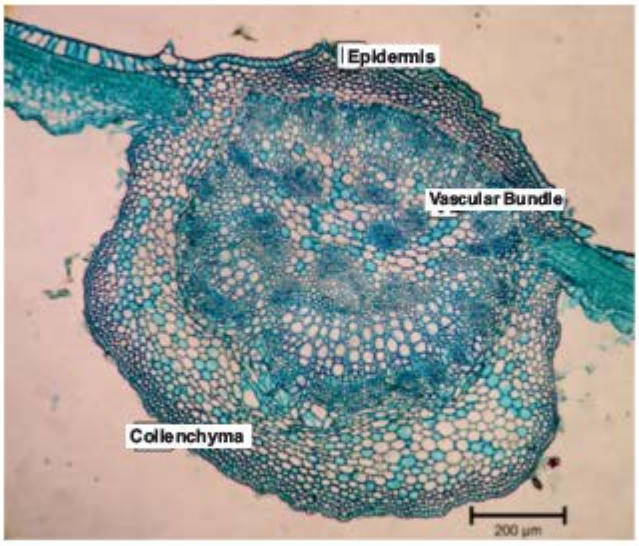


Plate 9

Adaxial Epidermis - A Portion Enlarged

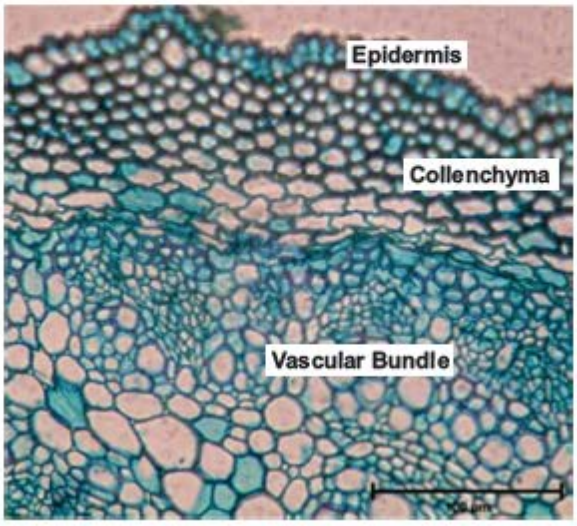


FIG - 3

T.S of Leaf of *T.grandis* through Midrib
Ground Plan

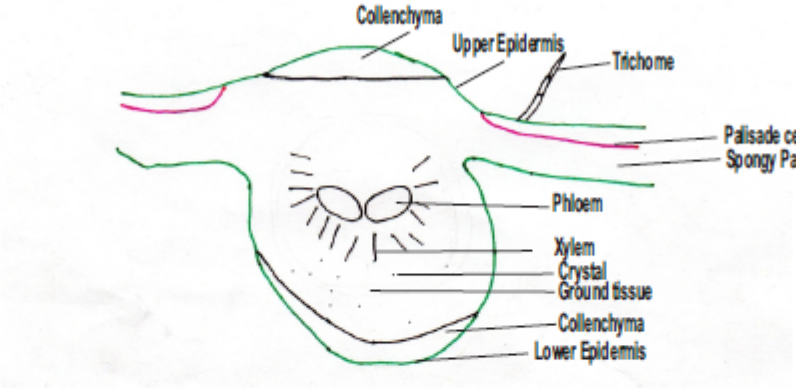


Diagram of T.S of leaf of *T.grandis* through Midrib

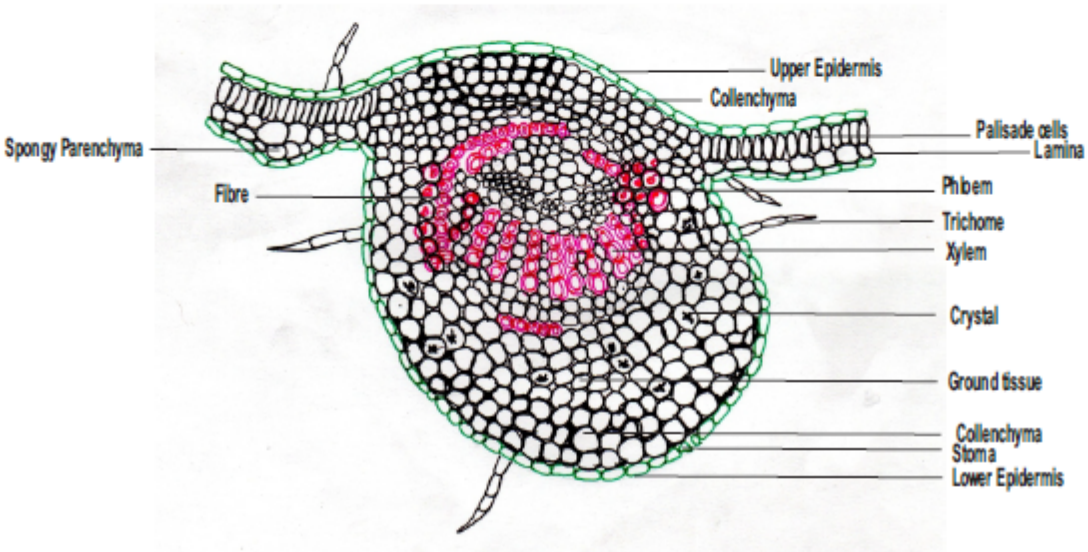


Plate 9-A

Adaxial Epidermis Surface View (Apostomatic)

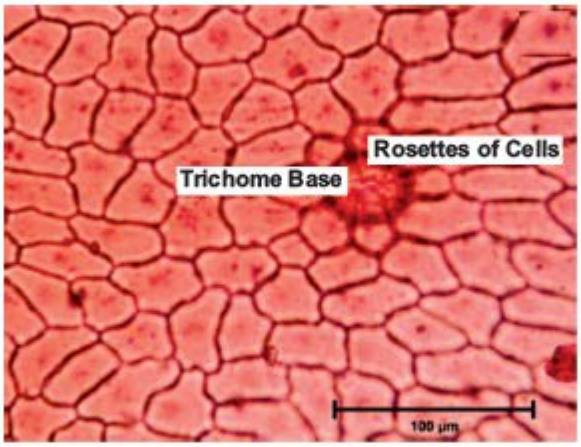


Plate 10

Abaxial Epidermis - A Portion Enlarged

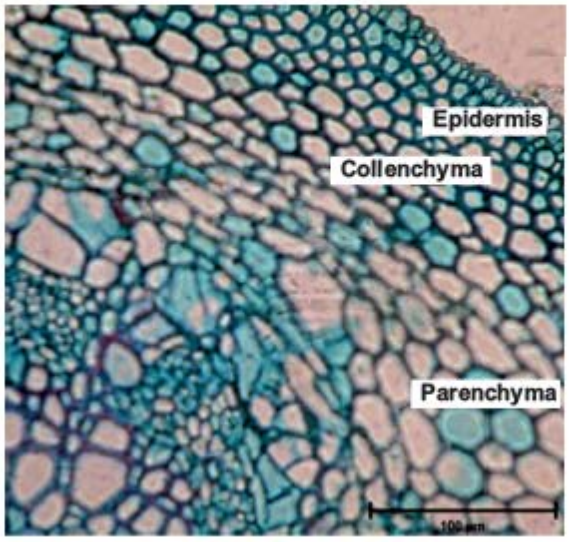


Plate 10-A

Abaxial Epidermis (Showing Stomata)

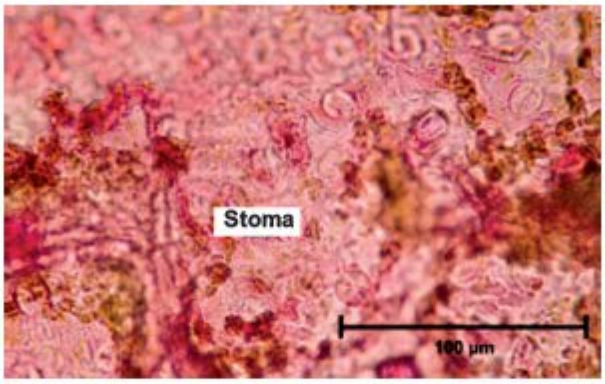


Plate 11

Vascular Bundle - A Portion Enlarged

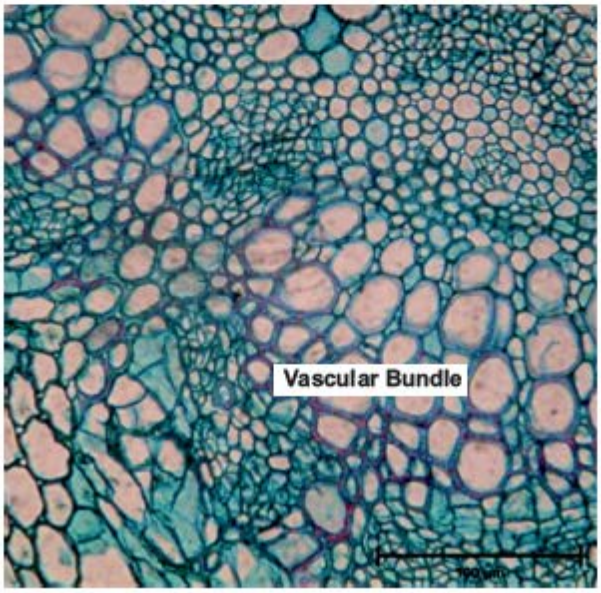


Plate 12

T.S. of Lamina - A Portion Enlarged

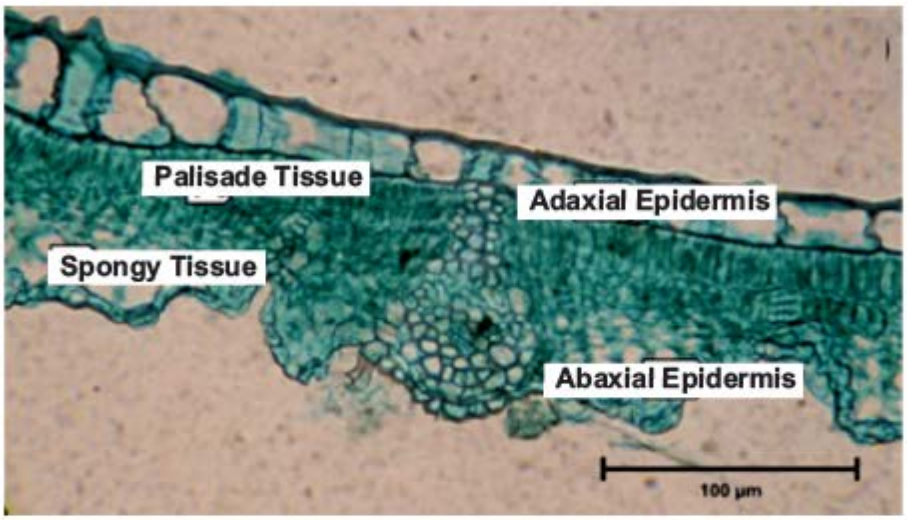


Plate 13

Venation Pattern

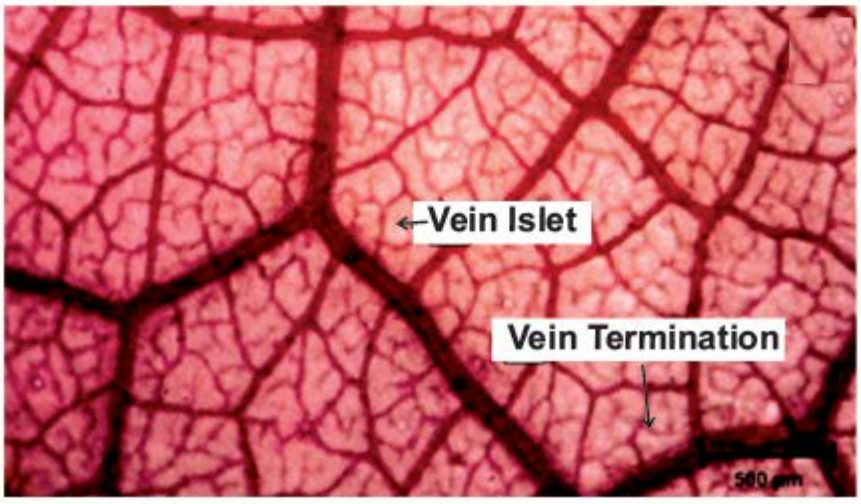


Plate 12

T.S. of Lamina - A Portion Enlarged

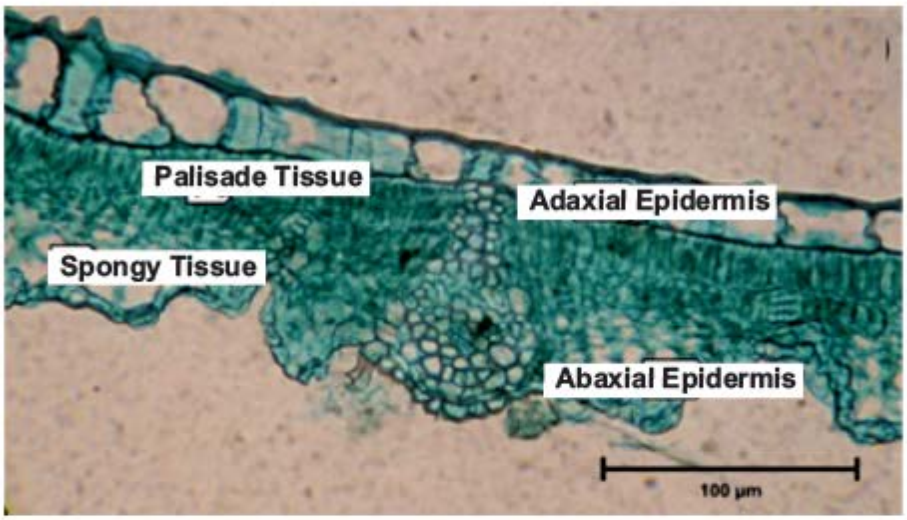


Plate 13

Venation Pattern

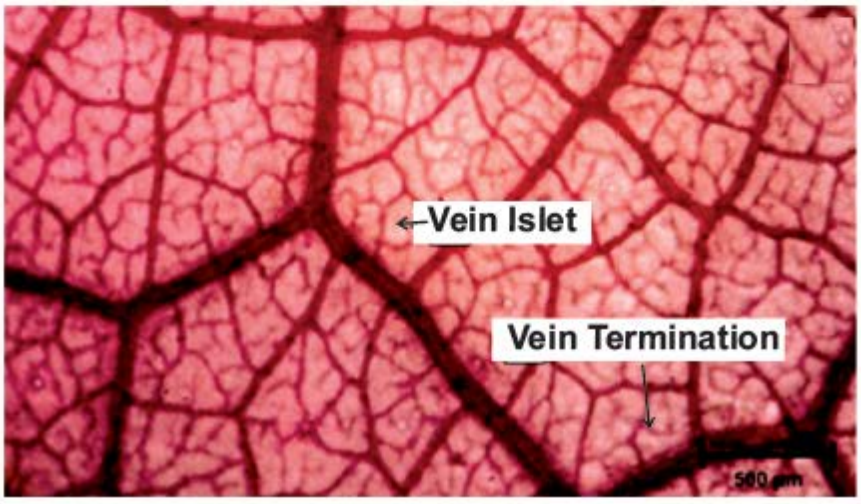
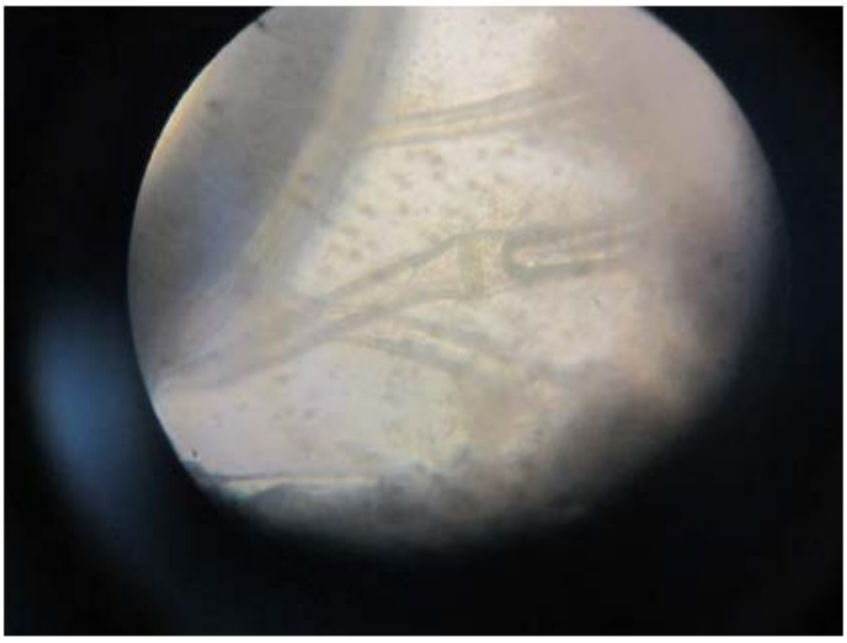


Plate 14

Trichomes of *T.grandis*

10 X



40 X

LEAF MIDRIB (Plate 8, Fig 3)

Shape : Plano convex

Adaxial side : Slightly raised round in shape,thick

Abaxial side : Semi circular broad and thick

Epidermis :

Adaxial epidermis:(Plate :9,9 A)

It is composed of larger cells and pentagonal in surface view with very slightly wavy contour.Apostomatic.It is covered by thick cuticle.

Abaxial epidermis: (Plate :10)

It is made up of smaller cells with wavy outline. Silicified rosettes of cellscontain cystolith like bodies are seen.

Stomata: (Plate : 10 A)

Deep sheatedranunculaceous stomata are present.

Hypodermis : (Plate : 9)

Hypodermal region composed of 3-6 rows of collenchyma cells.

Vascular Bundles (Plate : 8,11)

Large,situated in the centre and occupies the major portion of the midrib.It contains closed cylinder of xylem and surrounded by completely closed cylinder of phloem and fibres.

Ground Tissue : (Plate : 8)

It is made up of closely compactly packed parenchyma cells.Some cells contain cluster crystals of calcium oxalate.

Lamina: (Plate : 12)

Dorsiventral. The adaxial epidermis composed of larger and transversely elongated cells. Abaxial epidermal cells are rectangular and smaller in size. Mesophyll differentiated into palisade and spongy parenchyma. Palisade is composed of single layer of closely packed columnar parenchyma. Spongy mesophyll is composed of 2 or 3 layers of loosely arranged parenchyma cells.

Venation Pattern : (Plate : 13)

Distinct vein islets are formed by the secondary and tertiary veins.Vein islets are small and clearly seen;some of the vein terminations are forked.

Trichomes: (Plate :14)

It is numerous non-glandular hairs with cystolith in the cells surrounding the base.

T.S OF PETIOLE : (Plate : 15,16, Fig -4)

Shape:

More or less triangular with flat adaxial surface.

Epidermis:

Single layer made up of small rectangular cells covered by a striated cuticle.

Cortex:

It is divided into 2 zones with outer 6-8 rows of collenchyma cells and inner 10-15 rows of closely arranged polyhydral parenchyma cells.

Pericyclic Fibres:

2-3 layers of fibres seen as ring.

Plate 15

T.S of Petiole

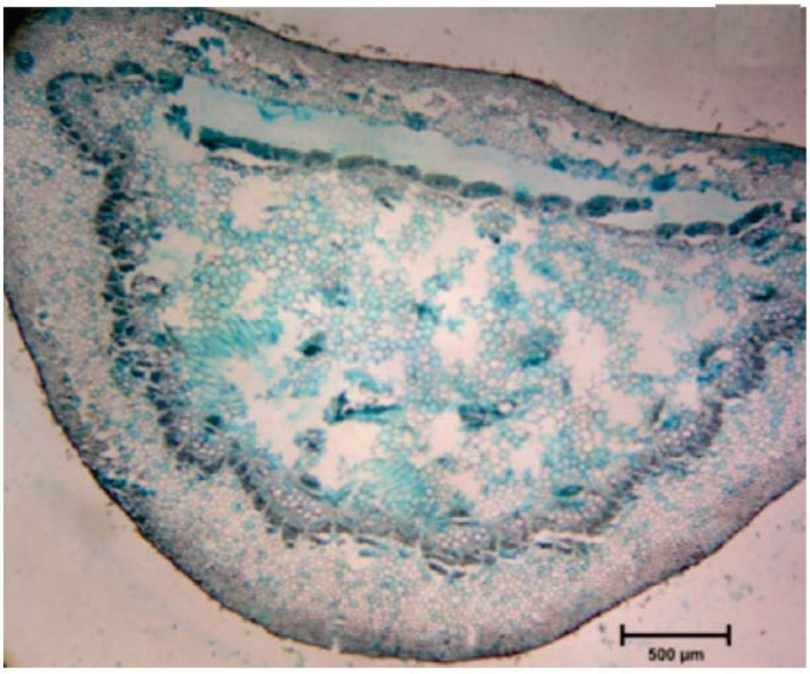


Plate 16

T.S of Petiole - A Portion Enlarged

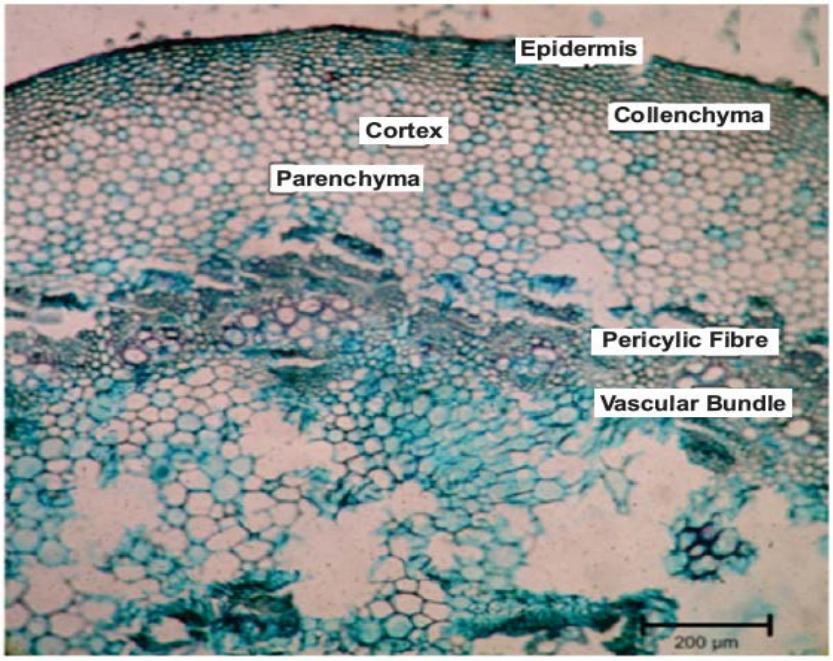
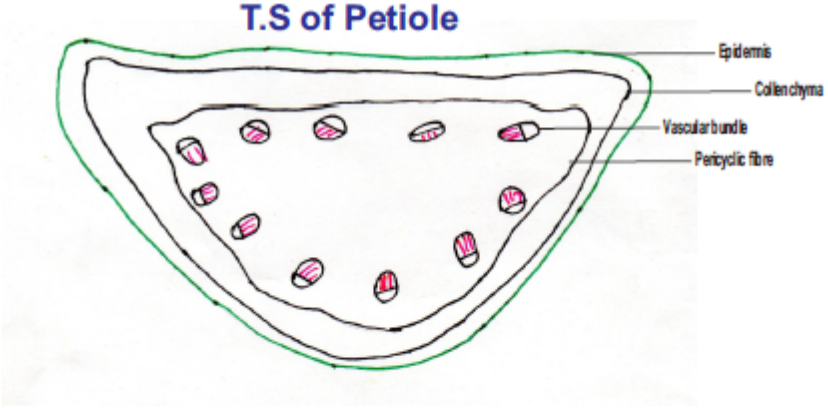
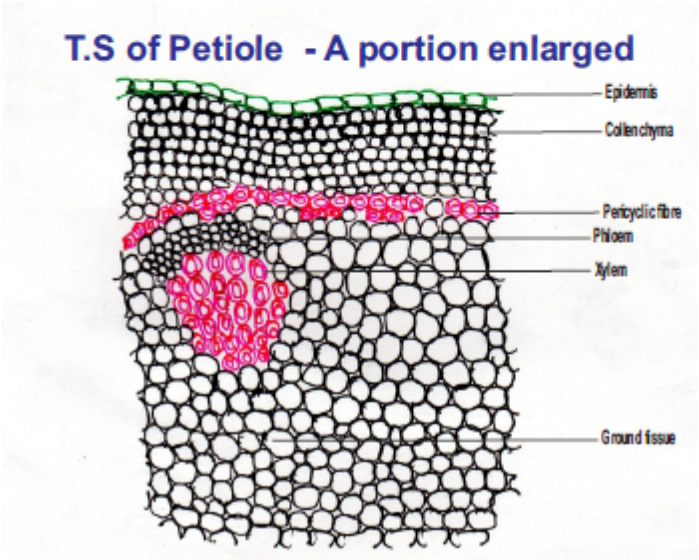


FIG - 4

T.S of Petiole



T.S of Petiole - A portion enlarged



Vascular Bundle:

It contain closed cylinder of xylem and phloem.

Pith:

Central large pith region composed of compactly arranged parenchyma cells.

5.1.3 SEM study of leaf (Plate 17,18,19,20)

Scanning Electron Microscopy study of the leaves showed no diagnostic feature and new kind of micro constituents not previously recognised and apparently simple struture which maybe extremely complex were observed.Trichomes,vessels,epidermis and other area studied in various magnifications.

5.1.4 POWDER MICROSCOPY (Fig -2)

Organoleptic characters

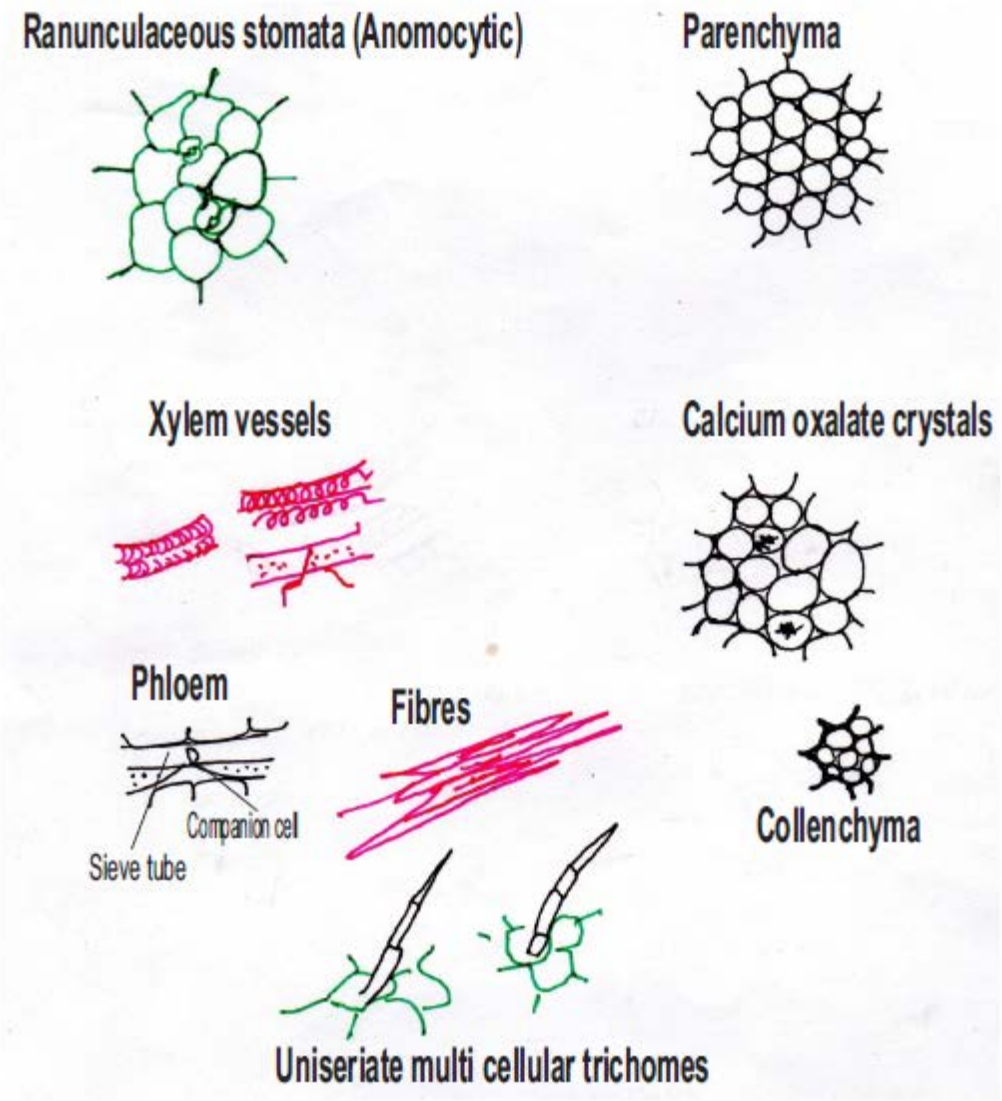
1. Nature : Coarse
2. Colour : Green
3. Odour : Characteristic
4. Taste : Characteristic,mild bitter
5. Shaken with water : Froathing occurs
6. Pressed in between two filter paper : No oil mark on the paper

We have observed the following microscopical cell structures,

- Ranunculaceous stomata(Anomocytic)
- Parenchyma
- Xylem vessels
- Calcium oxalate crystals
- Phloem
- Fibres
- Collenchyma
- Uniseriate multi cellular trichomes

FIG - 2

T.grandis Leaf Powder Microscopy



SCANNING ELECTRON MICROSCOPY

Plate 17

Trichomes - Various Magnifications

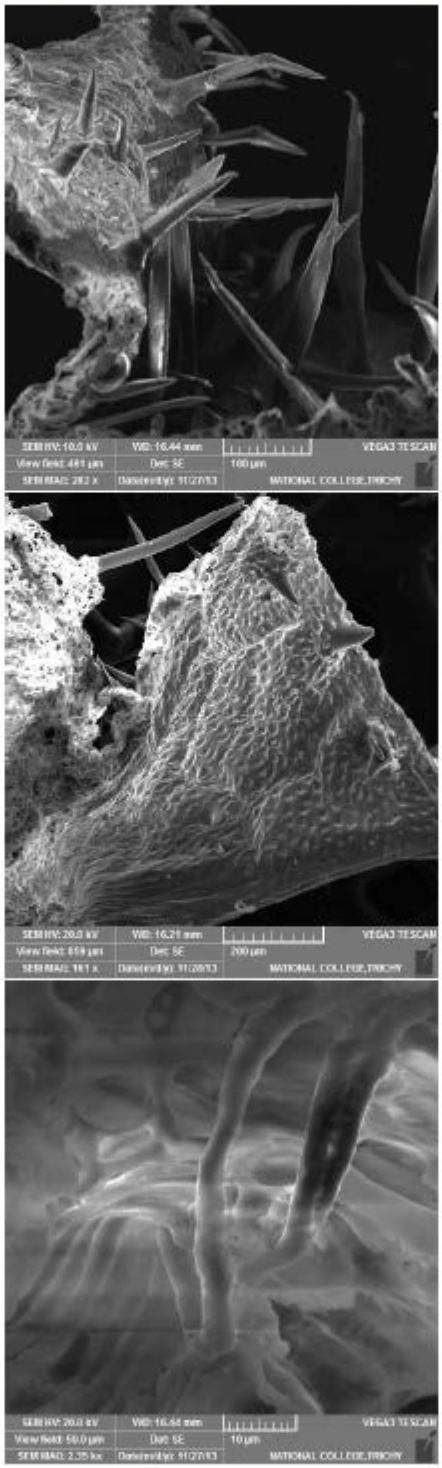
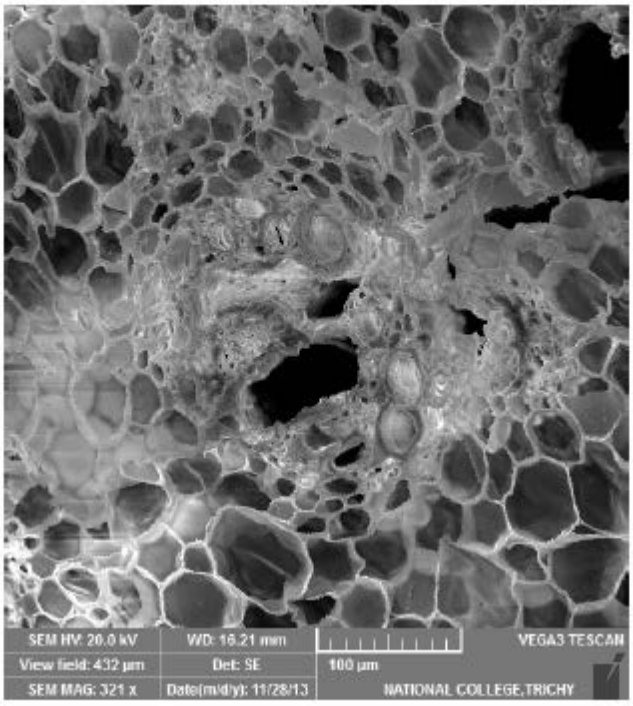


Plate 18

Vessels - Various Magnifications



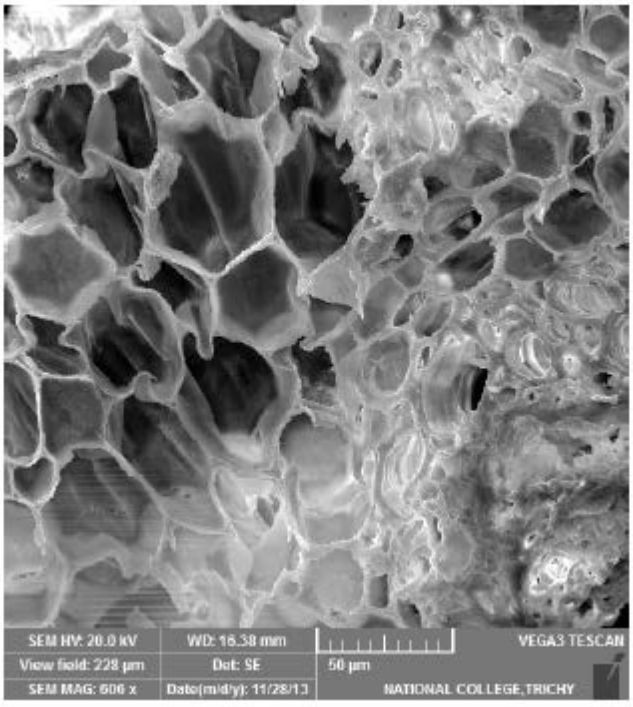


Plate 19

Lamina

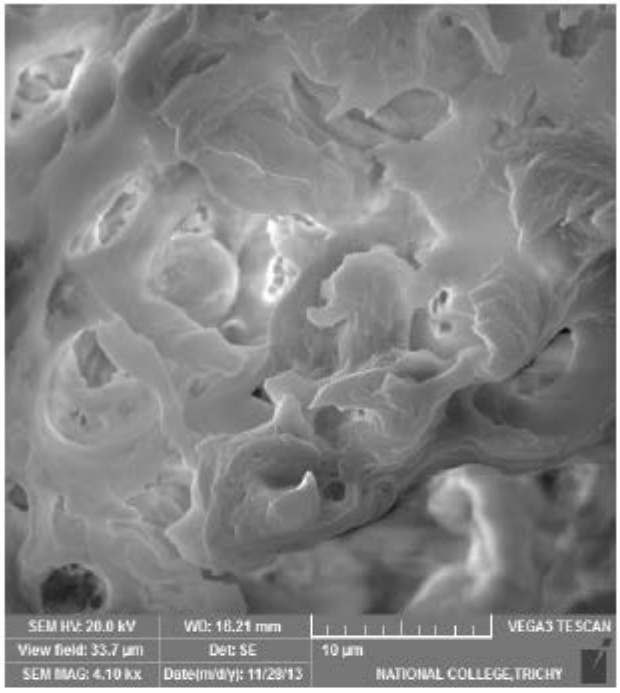
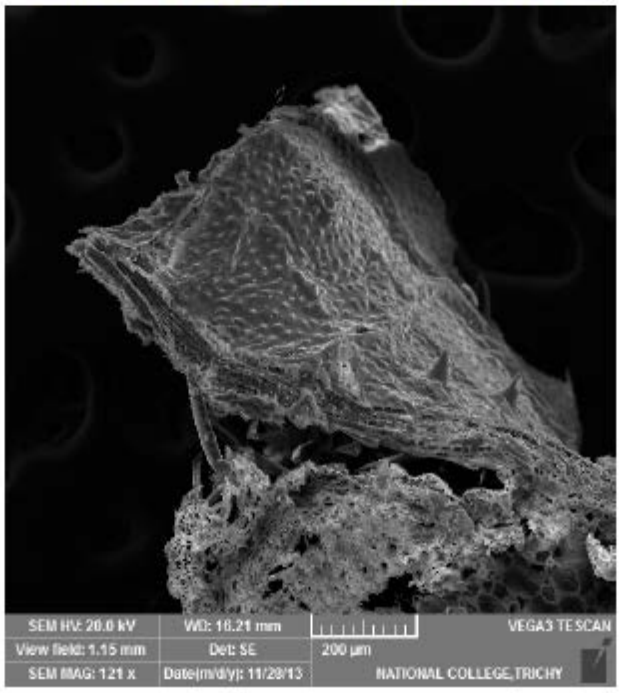


Plate 20

Upper Epidermis surface view



5.1.5 MICROSCOPIC SCHEDULES

As per the methods described in materials and methhods, microscopic schedules were carried out and the results tabulated from the Tables 1- 4.

Table – 1

VEIN ISLET AND VEIN TERMINATION NUMBER OF *T.grandis*LEAVES

Observation number	Vein Islet number	Vein termination number
1	26	9
2	27	7
3	30	6
4	32	5
5	31	8
6	28	9
7	34	8
8	29	6
9	33	7
10	28	6

Table – 2

Range	Minimum	Average	Maximum
Vein islet number	26	29.8	34
Vein termination	5	7.1	9

STOMATAL NUMBER OF *T.grandis*LEAVES

Observation number	Lower epidermis
1	65
2	60
3	62
4	66
5	61
6	67
7	64
8	59
9	63
10	55

Range	Minimum	Average	Maximum
Lower epidermis	55	62.2	67

Table – 3

STOMATAL INDEX OF *T.grandis*LEAVES

Observation number	Lower epidermis
1	31
2	34
3	30
4	32
5	36
6	33
7	34
8	32
9	31
10	35

Range	Minimum	Average	Maximum
Lower epidermis	30	32.8	36

Table – 4

PALISADE RATIO OF *T.grandis*LEAVES

Observation number	Palisade ratio
1	3
2	4
3	4
4	2
5	3
6	3
7	2
8	4
9	3
10	3

Minimum	Average	Maximum
2	3.125	4

5.1.6 PHYSICO CHEMICAL PARAMETERS

As per the methods described in materials and methods, physicochemical parameters were carried and the results were as follows

Table – 5

ASH VALUE OF THE LEAVES OF *T.grandis*

Observation Number	Total Ash (%)	Acid Insoluble Ash (%)	Water soluble Ash (%)
1	6..39	0.86	-
2	6.73	1.44	-
3	5.64	0.84	-
4	6.28	1.41	-
5	5.91	0.99	-
6	6.58	-	4.23
7	6.62	-	4.46
8	5.77	-	3.84
9	6.58	-	4.17
10	6.32	-	3.99
Minimum	5.64	0.84	3.84
Average	6.28	1.10	4.13
Maximum	6.73	1.44	4.46

Table – 6

LOSS ON DRYING (LOD) FOR *T.grandis*LEAVES

Observation number	LOD %w/w
1	0.63
2	1.02
3	1.13

4	0.68
5	1.07

Material	Minimum	Average	Maximum
Leaf powder	0.63	0.90	1.13

Table – 7

EXTRACTIVE VALUES FOR *T. Grandis*LEAVES(INDIVIDUAL SOLVENTS)

Solvents	Extractive value (%)
Petroleum ether	1.2
Ethanol	4.5
Water	5.1

Table – 8

EXTRACTIVE VALUESFOR *T. Grandis*LEAVES(SUCCESSIVE SOLVENTS)

Solvents	Extractive value (%)
Petroleum ether	1.21
Ethyl acetate	4.3
Ethanol	4.4
Water	5.12

5.2 PRELIMINARY PHYTOCHEMICAL SCREENING

5.2.1 Qualitative Phytochemical Test

Preliminary phytochemical screening of the powdered mature leaves were carried out and the results are as follows (Table 9)

TEST FOR ALKALOIDS

Mayer's test	:	No cream precipitate shows the **absence** of alkaloids
Dragendorff's test	:	Reddish brown precipitate shows the **absence** of alkaloids
Hager's test	:	No yellow precipitate shows the **absence** of alkaloids
Wagner's test	:	No reddish brown precipitate shows the **absence** of alkaloids
Muroxide test	:	No appearance of purple colour shows the **absence** of purine alkaloids

TEST FOR CARBOHYDRATES

Molish's test	:	Appearance of purple colour shows the **presence** of carbohydrates.
Fehling's test	:	Formation of reddish brown precipitate shows the **presence** of free reducing sugars.
Benedict's test	:	Formation of reddish brown precipitate shows the **presence** of free reducing sugars.

TEST FOR GLYCOSIDES

Keller killiani's test : No reddish brown colour ring at the junction shows the **absence** of cardiac glycosides.

Borntrager's test : Appearance of pink colour in ammonical layer shows the **presence** of anthraquinone glycosides

Modified Borntrager's test :

Appearance of pink colour in ammonical layer shows the **presence** of anthraquinone glycosides

TEST FOR PHYTOSTEROLS

Salkowski's test : Appearance of red colour in lower layer shows the **Presence** of sterol

Liebermann – Burchard's test : Brown ring at the junction of two layers and green colour in the upper layer shows the **Presence** of sterols

TEST FOR SAPONINS Frothing occurs indicates the **presence** of Saponins

TEST FOR TANNINS

Ferric chloride test : Appearance of bluish black colour shows the **presence** of tannins

Gold beater's skin : Appearance of brown colour shows the **presence** of tannins

TEST FOR PROTEINS AND FREE AMINOACIDS

Millon's test : Appearance of red colour on heating shows the

presence of proteins

Biuret test	:	Appearance of violet colour shows the **presence** of proteins
Ninhydrin test	:	Formation of violet colour shows the **presence** of amino acids
TEST FOR MUCILAGE	:	No appearance of reddish pink colour shows the **absence** of mucilage
TEST FOR TERPENOIDS	:	Appearance of pink colour shows the **presence** of terpenoids

TE ST FO R FL AV ON

OIDS

Shinoda test	:	Appearance of purple colour shows the **presence** of flavonoids
Alkaline reagent test	:	Appearance of yellow - orange colour shows the **presence** of flavonoids
Acid test	:	Appearance of yellow – orange colour shows the **presence** of flavonoids
Zinc hydrochloride test	:	Appearance of red colour shows the **presence** of flavonoids
TEST FOR VOLATILE OIL	:	Volatile oil not obtained shows the **absence** of volatile oil
TEST FOR FIXED OIL	:	No translucent greasy spot shows the **absence** of fixed oil

Table – 9

PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAVES OF *T.grandis*

S.NO	TEST	OBSERVATION
I.	**ALKALOIDS**	
	Mayer's reagent	-
	Dragondorff's reagent	-
	Hager's reagent	-
	Wagner's reagent	-
	Test for purine Group(Muroxide test)	-
II.	**CARBOHYDRATES**	
	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
III.	**GLYCOSIDES**	
	Anthroquinone glycosides	+
	Borntrager's test	+
	Modified Borndrager's test	+
	Cardiac glycosides	
	Keller Killiani test	-
	Raymond test	-
	Legal test	-
	Cyanogenetic glycosides	-
	Coumarin glycosides	-

IV.	**STEROLS**	
	Salkowski test	+
	LibermanBurchard’s test	+
V.	**SAPONINS**	+
VI.	**TANNINS**	
	Ferric chloride	+
	Gold Beater’s skin test	+
VII.	**PROTEINS AND FREE AMINO ACIDS**	
	Millon’s test	+
	Biuret test	+
	Ninhydrin test	+
VIII.	**MUCILAGE**	-
IX.	**TERPENOIDS**	+
X.	**FLAVONOIDS**	
	Shinoda test	+
	Alkali test	+
	Acid test	+
	Zn/Hcl test	+
XI.	**VOLATILE OIL**	-
XII.	**FIXED OIL**	-

5.2.2 FLUORESCENCE ANALYSIS OF POWDERED LEAF

The fluorescence analysis of the leaf powder of*T.grandis* was studied. The results were as follows (Table -10**)**

Table -10

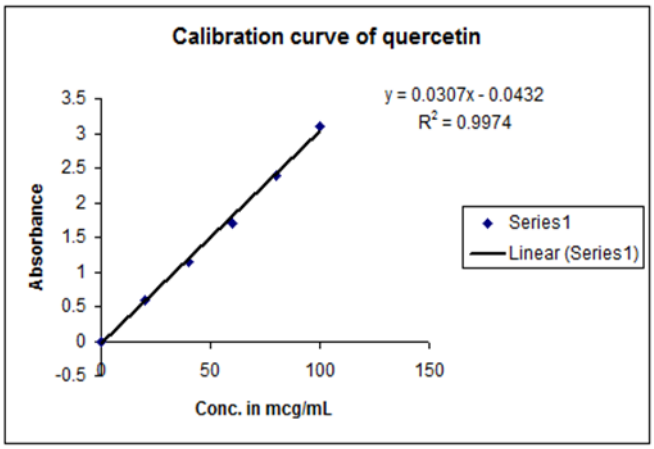
FLUORESCENCE ANALYSIS

Reagent	Observation
Powder as such	Faint green
Powder + 50% Hydrochloric acid	Yellowish Green
Powder + 50% Nitric acid	Light brown
Powder + Petroleum ether	Light orange
Powder + 50% Sulphuric acid	Dark brown
Powder + 1N NaOH in water	Dark green
Powder + 1N NaOH in methanol	Dark Green
Powder +5% Ferric chloride solution	Greenish brown
Powder + Picric acid	Flourescence green
Powder + Chloroform	Green
Powder + 5% Iodine solution	Green
Powder + (HNO3 + NH3)	Green

5.2.3 Estimation of flavonoid content of TGEAE

Figure: 5

Calibration curve of quercetin for estimation of flavonoid content



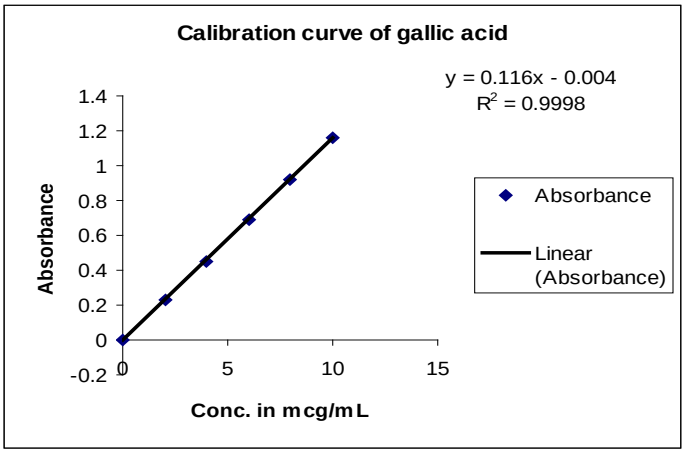
Report:

Flavonoid content of TGEAE in terms of quercetin by aluminium chloride was found to be 13.5µg/g.

5.2.4 Estimation of Total phenolic content of TGEAE

Figure :6

Calibration curve of gallic acid for estimation of total phenolic content



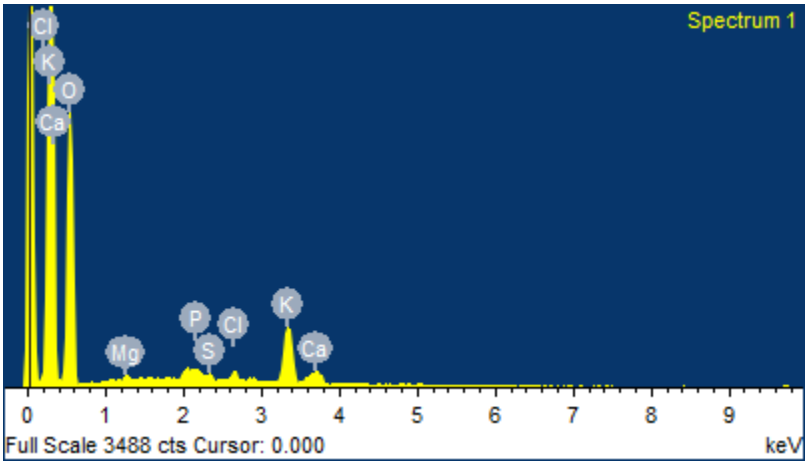
Report:

Total phenolic content of TGEAE in terms of gallicacid was found to be 22.2µg/g.

5.2.5 IDENTIFICATION OF INORGANIC MINERALS OF THE LEAVES OF *T.grandis* by Energy Dispersive X-ray Spectrometer (EDS)

Figure-7

Energy Dispersive X-Ray Spectrum For *T.grandis*Leaves



Estimation of the elements like O, Mg, P, S, Cl, K, Ca, Zn showed the following mg weight percentage and atomic percentage.

Table-11
***T. grandis*Leaves Elements weight & Atomic Percentage**

SI.NO	ELEMENTS	WEIGHT(%)	ATOMIC(%)
1.	OK	85.25	93.05
2.	MgK	0.62	0.45
3.	PK	0.97	0.55
4.	SK	0.48	0.26
5.	ClK	1.24	0.61
6.	KK	9.24	4.13
7.	CaK	2.19	0.96

5.2.6. HPTLC PROFILE OF THE TGEAE &TGEE OF THE LEAVES

Development of HPTLC fingerprint

About 2,6,8,10 µl of standard Apigenin ,4,8 µl of TGEAE,3 µl Quercetin,6µl of TGEE was applied as a band using CAMAG Linomat sample applicator on aluminium sheets pre-coated with silica gel 60 GF 254 HPTLC plates used as a stationary phase. The plates were developed in the mobile phase Toluene : Ethyl acetate: Formic acid : Methanol (3:6:1.6:0.4) to a distance of 8cm in CAMAG-twin trough glass chamber. The tracks were scanned using WIN CATS 1.43 software at 254nm. The fingerprint profiles were recorded and presented in **Table-13**

Plate size : HPTLC plates silica gel 60 F 254

(E.MERCK KGaA)

Sample solvent type : Methanol

Dosage speed : 150 nl/s

Number of tracks : 8

Table-12

HPTLC profile of the TGEAE & TGEE leaves

NO	Appl.position	Appl.volume	Sample ID	Active
>1	10.0 mm	2.0 µl	API	Yes
>2	21.4 mm	6.0 µl	API	Yes
>3	32.8 mm	8.0 µl	API	Yes
>4	44.2 mm	10.0 µl	API	Yes
>5	55.6 mm	4.0 µl	TGEAE	Yes
>6	67.0 mm	8.0 µl	TGEAE	Yes
>7	78.4 mm	3.0 µl	QUERCETIN	Yes
>8	89.8 mm	6.0 µl	TGEE	Yes

Chamber type : Twin Trough Chamber 10×10cm

Mobile phase : TOLUENE : ETHYL ACETATE : FORMIC

ACID : METHANOL (3:6:1.6:0.4)

Drying device : Oven

Temperature : 60^{o} C

Time : 5 Minutes

Detection : CAMAG TLC Scanner 3

Scanning speed : 20 mm/s

Wavelength : 254

Lamp : D2 &W

Measurement Type : Remission

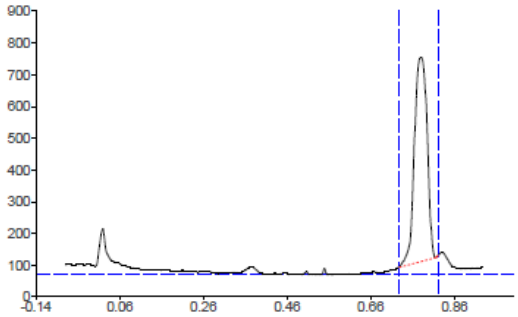
Measurement Mode : Absorption

Detector mode : Automatic

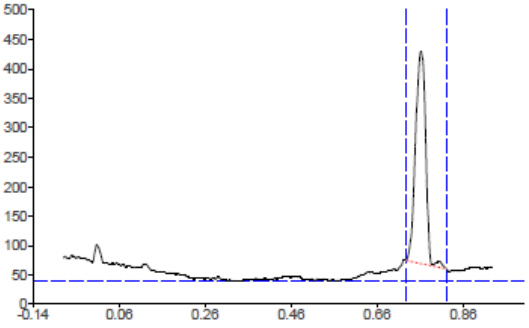
Figure-9

HPTLC graph of TGEAE & TGEE of the leaves

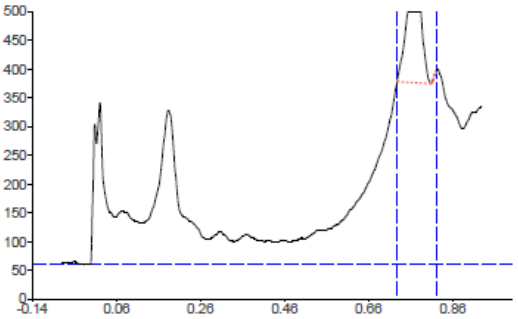
STANDARD : APIGENIN



STANDARD : QUERCETIN



SAMPLE: TGEAE



SAMPLE : TGEE

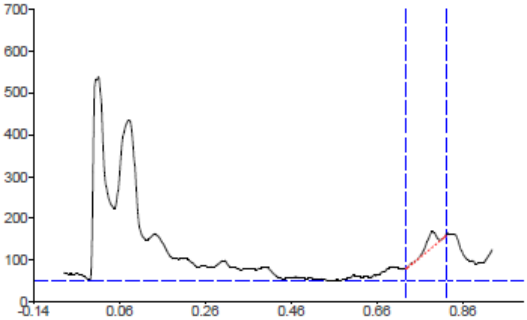


Table – 13

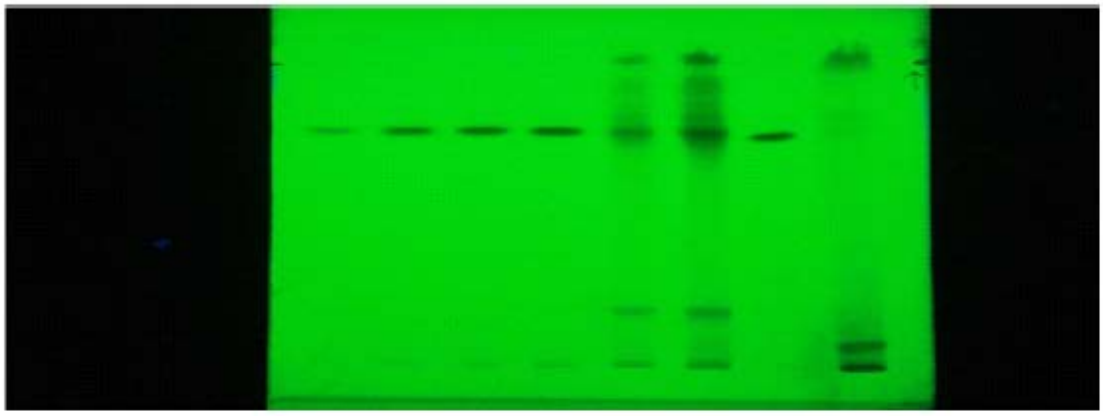
Rf values for TGEAE & TGEE leaves

SI NO	SAMPLES	Start Rf	Max Rf	End Rf	Area	Area %
1	Standard Apigenin	0.73	0.78	0.81	15860.2	100.00
2	Sample TGEAE	0.73	0.77	0.81	5696.2	100.00
3	Standard Quercetin	0.73	0.76	0.79	6814.8	100.00

4	Sample TGEE	0.75	0.79	0.81	662.3	100.00

Plate 21

HPTLC PLATES OF TGEAE AND TGEE OF THE LEAVES UNDER UV 254nm



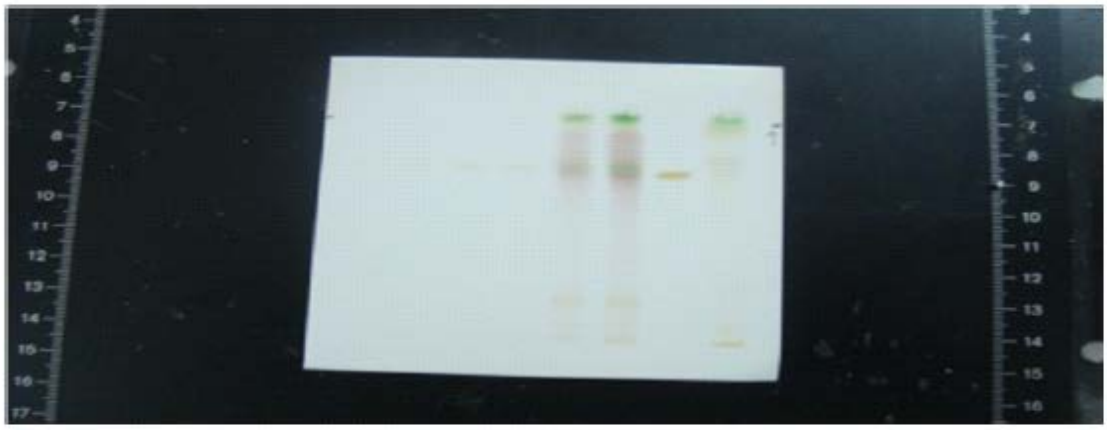
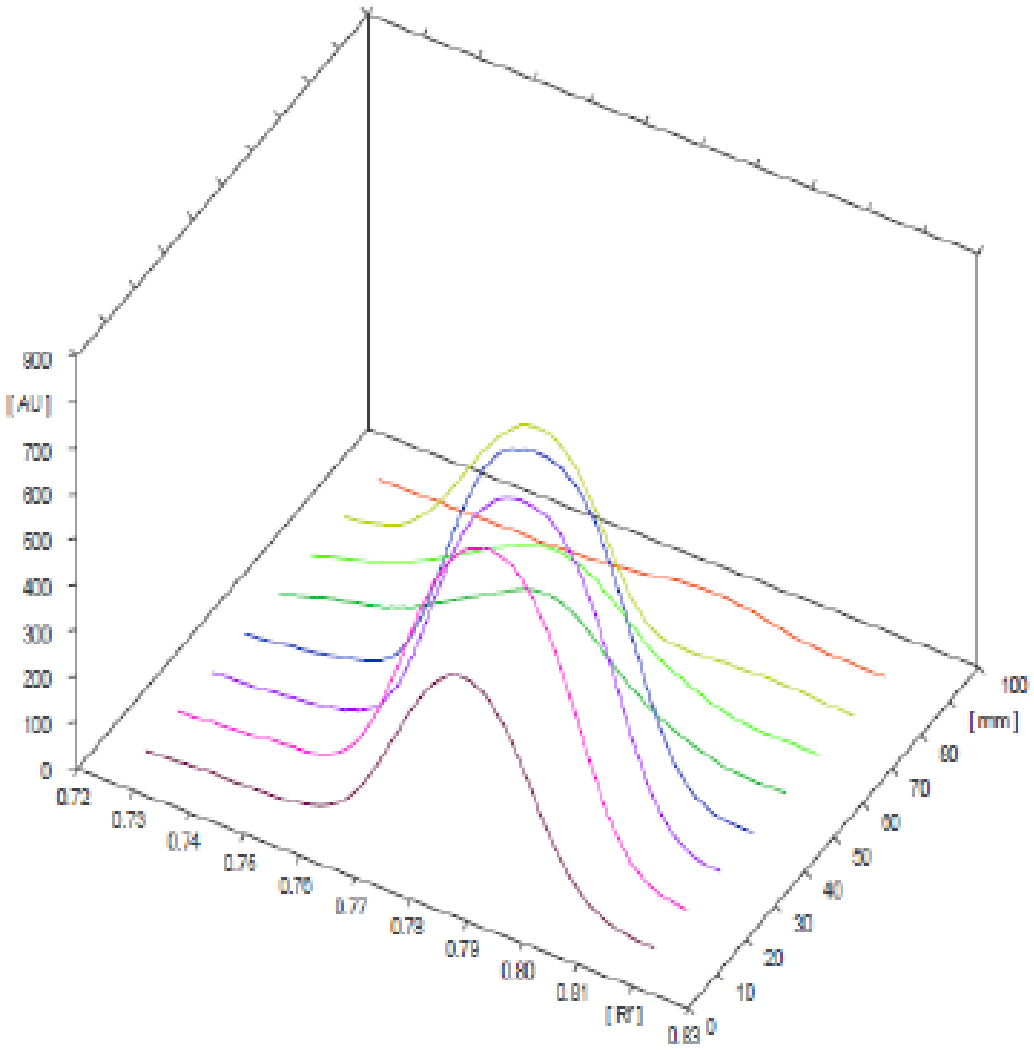


FIG-8

HPTLC PROFILE OF THE TGEAE AND TGEE OF THE LEAVES



5.3 .PHARMACOLOGICAL STUDIES

5.3.1.Acute toxicological study

EFFECT OF VARIOUS CONCENTRATIONS OF ETHYL ACETATE EXTRACT FROM THE LEAVES OF *T.grandis*L ON *Artemianauplii*

Table-14

Various Concentrations Of TGEAE leaves on *Artemianauplii*

Concentration (ppm)	Number of Larvae released	Number of Larvae dead after 24hrs	Mortality (%)	Corrected (%) Mortality using Abbot's Formula
100	10	2	20	20
	10	2	20	
	10	2	20	
200	10	2	20	20
	10	2	20	
	10	3	20	
500	10	4	40	36.6
	10	4	40	
	10	3	30	
1000	10	4	40	43.3
	10	5	50	
	10	4	40	

2500	10	3	30	43.3
	10	5	50	
	10	5	50	
5000	10	5	50	50.0
	10	5	50	
	10	5	50	
6000	10	6	60	63.3
	10	7	70	
	10	6	60	
7500	10	7	70	66.6
	10	7	70	
	10	6	60	
Control	10	-	-	-
	10	-	-	
	10	-	-	

5.3.2 *In-vitro* Anti-Malarial Activity of TGEAE of The Leaves

Anti-malarial activity of the ethyl acetate extract *T.grandis* against *P.falciparum.* (Plate-22)

After the incubation the percentage of parasitemia in the control well has increased drastically after 96 hrs as compared to the parasite at 0 hrs.This increase shows the normal growth of the parasite in the RBC's.The percentage of increase of the parasite at 48 hrs,72 hrs and 96 hrs have been tabulated(Table-16).

In the standard wells, a 100% reduction was noticed during 48 hrs,72hrs and 96hrs which indicates the action of the known anti-malarial drug chloroquine against the parasite.

In the test the percentage of reduction of the parasite at 48 hrs is 33%,72 hrs is 25% and 96 hrs is 18%.This showed that ethanol extract of leaves of*T.grandis*induced a significant decrease of *P.falciparum* proliferation.

This shows it is an inexpensive and affordable crude extract and can be evaluated further for formulations.

Fig-10

Anti–malarial activity of TGEAE of the leaves

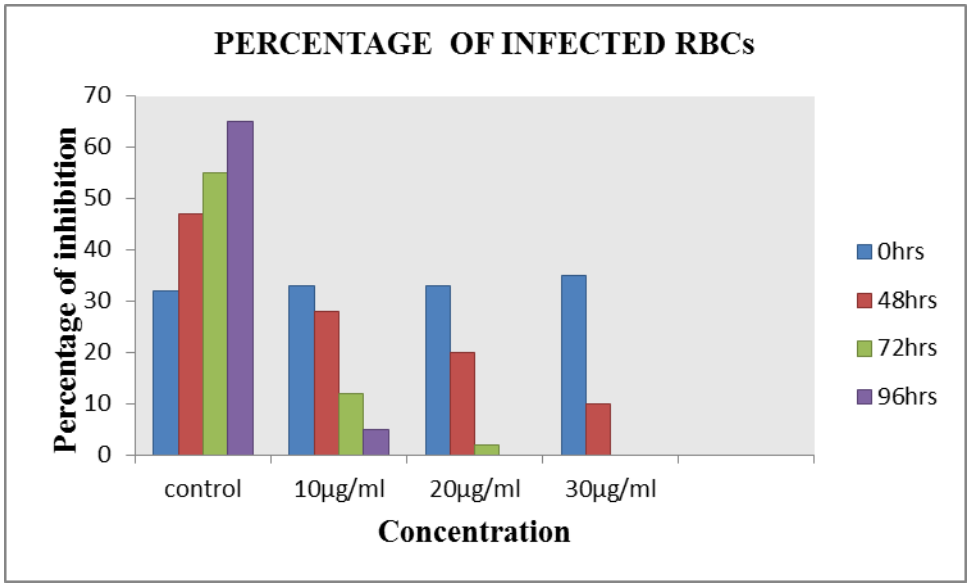
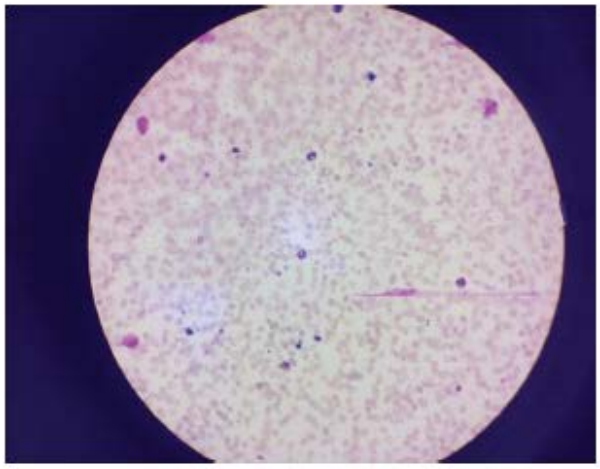
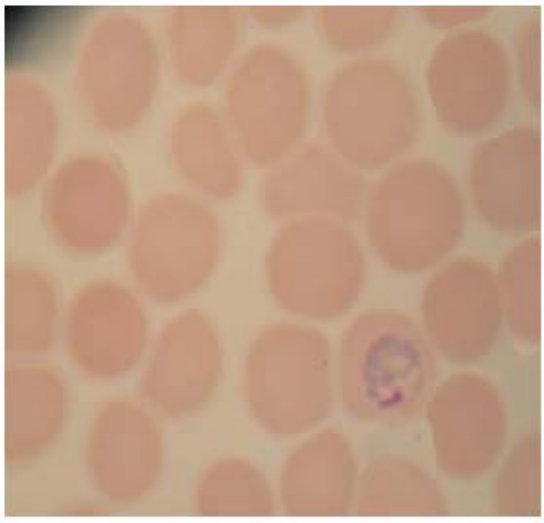


Plate 22

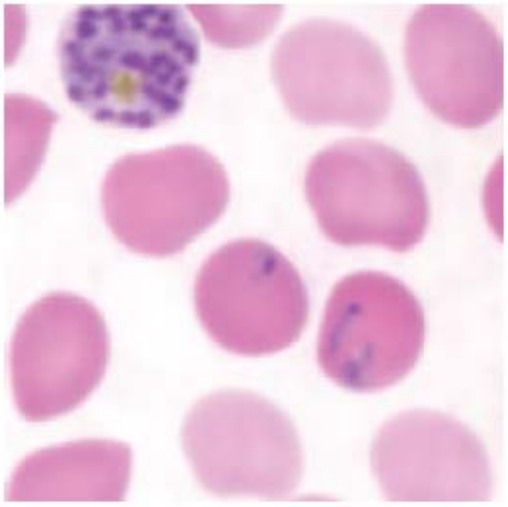
Plasmodium infected RBC under low power



Plasmodium infected RBC under high power



***P.falciparum* schizont**



RBCs infected at different stages

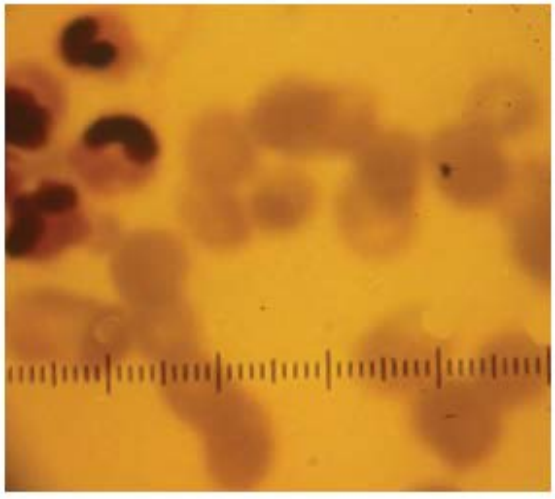


Table-15
PERCENTAGE OF INFECTED RBC's

Sl No	Samples		0hrs	48 hrs	72 hrs	96 hrs
1	Control		32±1.3	47±1.5	55±1.3	65±0.9
2	Test	10 µg/ml	33±1.12	28±0.85	12±1.12	5±0.79
3		20µg/ml	33±0.96	20±1.12	2±1.3	NIL
4		30µg/ml	35±1.1	NIL	NIL	NIL

5.3.3.EFFECT OF TGEAE OF THE LEAVES ON *ex-vivo* PORCINE SKIN WOUND HEALING MODEL:

Wound healing effect was evaluated by *ex-vivo* porcine skin wound healing model (PSWHM). Architecture of pig skin is closer to human (Bollen Peter JA *et al*, 1999, Laber*et al*, 2002). Porcine model is an excellent tool for the evaluation of therapeutic agent meant for wound healing (Sullivan TP *et al*, 2001).

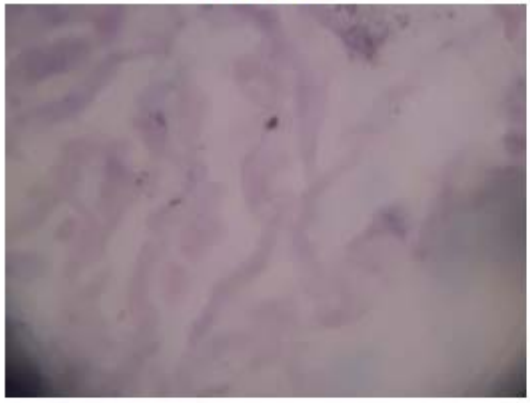
Histopathological evaluation has showed all treated wounds were sound with no signs of apoptosis, necrosis or bacterial contamination and no toxicity of the tested concentrations of TGEAE of the leaves.(Plate). Morphology of the wound margins, epidermis and dermis layer were found to be normal. Epidermal cell migration in each side of the wound was measured from the edge of the wound to the migration tip.

Measurement of epidermal migration distances from the wound showed statistically significant dose dependent wound healing effect (Anova $p<0.05$).The efficacy of wound healing promoting effect was expressed as normalised epidermal migration % mean SE against PBS control groups.(Fig 10)

Plate 23

Histology showing effect of TGEAE on ex-vivo PSWHM

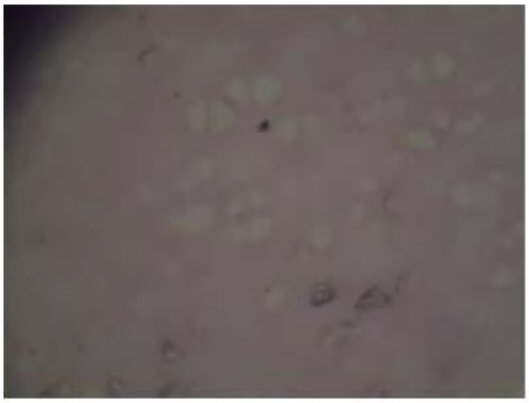
Control



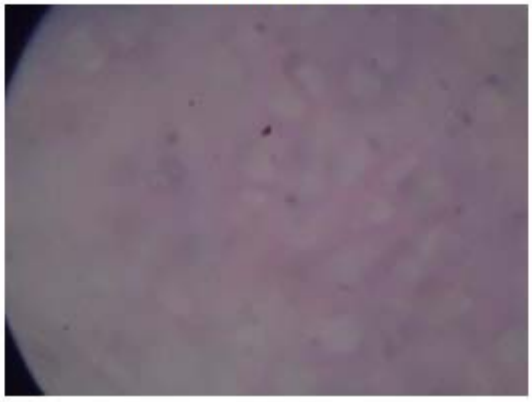
1%

2%

3%



Standard



CHAPTER-6

DISCUSSION

The dissertation covers a study on widely available member of the family Verbenaceae known botanically as *Tectonagrandis*Linn.commonly called as 'Teak'. The leaves of *T.grandis* really do not have any match as a cheap natural and easily available plant.It is traditionally known to be useful for the treatment of wide panel of diseases like tuberculosis, various kinds of **wounds** especially burn wound, **malaria, anaemia**, leprosy, skin diseases like pruritis, stomatitis, indolent ulcers, haemorrhages, menstrual disorder, bone joint disease. Young leaves and fruits prescribed for women for fertility control and fumes of dry leaves to kill guinea worms.It also possesses anti- ulcer and anti- cancer activity. Various scientific investigation of the leaves showed anti-diabetic activity, anti-microbial activity, anti-oxidant, analgesic, anti-inflammatory,anti-amoebic, anti-hypertensive and wound healing activity.

Bark is used as astringent, expectorant, anti-inflammatory, anthelmintic and for constipation, leucoderma,headache, piles, bronchitis, hyperacidity, and dysentery,burning sensation, diabetes, difficult in labour, leprosy, skin diseases, and indigestion.Researches proved it is anti-fungal, anti-bacterial, uterine relaxant, anti-histaminic, anti-asthmatic, anti-oxidant, analgesic and anti-inflammatory, anti-diabetic, hepatoprotective and cardiac activities.

Wood traditionally used as diuretic, stimulant, hepatic astringent, toothaches, sedative to gravid uterus,leucoderma.It was reported that wood possesses anti-oxidant,anti-termite, anti-hyperglycaemic, nephroprotective activities.

Flowers used for bronchitis, biliousness, urinary discharge, diuretic, depurative, anti-inflammatory, leprosy, skin diseases, diabetes, congestion of liver.

Seed is used in poisoning.Seed oil in skin disease.Oil obtained from seeds and flowers promote the growth of hair,eczema,ring worm, scabies. Scientific investigation of seed extract proved hair growth activity, diuretic, anti-inflammatory activity.

Root is useful in anuria and retention of urine, anaemia. Anti-ulcer activity, anti-inflammatory, anti-tussive, anti-oxidant, hypoglycemic, anti-pyretic activities have been evaluated and proved.

The economic aspect of this crop evidently proved that as commercial crop. In fact the revenue generated by this crop can be further magnified by many folds, if its medicinal applications are scientifically explored well. By a well-coordinated effort, we can properly exploit this plant. Therefore research on development of herbal products from this plant is required to be initiated immediately for exploring the unique potential of this crop which would also minimize the menacing wastage especially the leaves. It may be further envisaged that the revenue generated by this plant would easily exceed that generated by any major crop of the country even with a present level of traditional agro economic practices. Therefore a well-coordinated effort by the farmers, traders, scientist, technologists, extension workers, physician, administrators, and policy makers is required to be initiated to boost up the national economy as well as the proper exploitation of this for proper therapeutic purpose. The review of literature showed some lacuna exists in the pharmacological, phytochemical, and pharmacological studies in the leaves of *T. grandis*.

PHARMACOGNOSTICAL STUDIES:

Morphological and micromorphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacological studies. There was no detailed pharmacognostical work has been carried out including botanical identity based on micro morphology in this leaves of this plant.

The application of morphological studies in drug analysis is pertinent in the field of crude drug authentication. It was studied for the leaf. Interpretation of the morphological characteristics based on different parameters, for the plant organs give a guideline for the diagnosis of the original plant and its adulterants.

Colour, size, shape, margin, texture, arrangement were observed and compared with previous data.

Microscopic techniques help to magnify the fine structure of minute objects and there by confirm the structural details of the plant drug. Though the microscopical evaluation cannot provide complete profile, still it can offer supporting evidences which when combined with other analytical parameters can be used to obtain full evidence for standardization and evaluation of herbal drugs. Consideration must therefore be given to the types of cells and cell inclusions and the manner in which they are distributed in different organ of the plants. The habit and habitat and the various morphological characters of the various parts have been studied after proper identification and authentication.

PHARMACOGNOSTICAL STUDIES (Plate : 8-14)

The leaves are dorsiventral elliptic or obovate or acuminate.The upper surface is rough, glabrous, the lower clothed with dense stellate grey or tawny tomentum petiolate, opposite or whorled, large entire,Length 30-60 cm ×20-30 cm green entire margin with cuneate base.

In microscopicaltransectional view plano convex with slightly raised round thick adaxial side and semi-circular broad thick abaxialside.The upper epidermis apostomatic with larger cells and pentagonal surface view with very slightly wavy walls but the abaxial epidermis is made up of smaller cells with wavy outline with frequent silicified rosettes of cells containing cystolith like body. It contains ranunculaceous stomata, collenchyma cells is

present in the hypodermal region of hump and lower epidermis. The lamina contain single layer of palisade and spongy parenchyma. The vascular bundle situated in the centre of midrib contains closed cylinder of xylem surrounded by phloem and fibres. The ground tissue is made up of compactly packed parenchyma cells with frequent calcium oxalate crystals. Numerous non-glandular hairs with cystolith were observed. Some distinct vein islets and vein terminals(some are forked) were clearly seen.

The petiole more or less triangular with flat adaxial surface. The epidermis is covered with striated cuticle. Cortex contain outer collenchyma cells and inner polyhydral parenchyma cells. Vascular bundle contain xylem and phloem fibres. Central large pith region composed of parenchyma is present.**(Plate : 15,16)**

Scanning electron microscopy (SEM) study showed no diagnostic features and new kind of micro-constituents not previously recognized and apparently simple structure which may be extremely complex were observed.**(Plate : 17-20)**

The plant drugs are generally used in the powdered form where the macro morphology is generally destroyed, so the diagnosis of the plant through the microscopicalcharacter is essential. The powdered crude drugs can be identified based on the presence or absence of different cell types.

In powdered microscopy observed ranunculaceous stomata, wavy walled epidermal cells, spiral annular vessels, phloem cells, sieve tube and companion cells, fibres, collenchyma cells, parenchyma cells, calcium oxalate crystals.**(Fig-2)**

Quantitative microscopy includes certain measurements to distinguish some closely related species which are not easily differentiated by general microscopy. The **stomatal number** is

the oldest technique but a simple method of diagnosis of fragmentary leaf parts. The **stomatal index** is the percentage of stomata in relation to the epidermal cells. Both are very specific criteria for the identification and characterization of leafy drugs. **Vein islet and vein terminationnumber** are another simple technique for distinguishing fragmentary specimens at specific levels. It is used as the distinguishing character for the leaf of the same species or different one.

Palisade Ratio is another criterion for identification and evaluation of herbal drugs. This value remains constant within a range for a given plant species and is of diagnostic value in differentiating the species. This value does not alter based on geographical variation and differs from species to species and that is why it is a very useful diagnostic feature for characterization and identification of different plant species. **(Table 1-4)**

The ash content of the crude drug is generally taken to be the residue remaining after incineration. If usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also involve the inorganic matter added for the purpose of adulteration. There is a considerable difference within narrow limits in the case of individual drug. Hence ash determination furnishes a basis for judging the identify and cleanliness of a drug and gives information related to its adulteration with inorganic matter. The ash or residue yielded by an organic chemical compound is a rule to measure the amount of inorganic matter, which is present as impurity. In most cases the inorganic matter is present in small amounts which are difficult to remove in the purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drug in especially in powdered from. The **acid insoluble ash** is of more value to detect the earthy matter adhering to the drug. In this way one can obtain evidence of the presence of

foreign matter, which likely to occur with root, rhizomes and also in pubescent leaves. **The water soluble ash** is used to detect the presence of matter exhausted by water. Insufficient drying favours spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles **(Table -5).**

Extractive values of crude drugs determine the amount of active constituents in a given amount of medicinal plant material when exhausted with solvents. It is employed for that material for which no chemical or biological assay method exist. As mentioned in different official books [Anonymous., 1996, Anonymous.,2006,Horborne JB.,1973] the determination of water-soluble and alcohol soluble extractive, is used as means of evaluating crude drugs which are not readily estimated by other means. The extraction of any crude with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used. The use of single solvent can be the means of providing preliminary information on the quality of a particular drug sample. The **water soluble extractive** values play an important role for the evaluation of crude drugs. It can be used to indicate poor quality, adulteration with any unwanted material or incorrect processing of the crude drug during the drying, storage etc. The **alcohol soluble extractive** is also indicative for the same purpose as water soluble extractive values **(Table -7, 8).**

Loss on drying at 105^{o}C is determined as the presence of excess moisture is conductive to the promotion of mold and bacterial growth, and subsequently to deterioration and spoilage of the drug **(Table -6)**

THE PRELIMINARY PHYTOCHEMICAL STUDIES:

The preliminary phytochemical screening reveals the presence of carbohydrates, proteins and amino acids,anthraquinoneglycosides, flavonoids, terpenoids, tannins, saponins, sterols. Alkaloids,Volatile oil, fixed oil were found to be absent. **(Table -9)**

The reaction of drugs in powdered form in ordinary light and with filtered UV light is of importance in several cases by the luminosity in UV light by **fluorescent analysis**. Many flavonoids showed distinctive colours under UV light: Bright yellow (6-hydroxy flavanoids and flavones and some chalcones), dark brown (most flavanol glycosides, dark may be (isoflavones and flavonols). Hence this parameter can also be used as a diagnostic tool for the standardization of herbal drugs for the detection of adulterants in crude drugs **(Table-10)** (Horborne JB., 1973).

Determination of total flavonoid content was found to be 13.5µg/g in TGEAE.Determination of total phenolic content was found to be 22.2µg/g in TGEAE.

Identification of inorganic minerals of the leaves of *T.grandis*byenergy dispersive X-ray spectrometer(EDS)showed the presence of mineralsoxygen(85.25%),magnesium(0.62%),phosphorous(0.97%),sulphur(0.48%),chlorine(1.24%),potassium(9.24%),calcium(2.19%)**(Table-13)**. No special characters were observed in SEM analysis.

It was reported that essential oils, phenolic compounds, iridoids, triterpenes were isolated from the different member of the Verbenaceaefamily. In an investigation presence of quercetin, apigenin, gallic acid, quercetrin, chlorogenic acid, diosmin, kaempferol, hesperidin etc. in the ethylacetate extract of the leaves have been reported[Ghareeb MA *et al.*,2013]. In the therapeutically important constituents apigenin, and other flavonoids may be responsible for the various pharmacological activity of the leaf. In an attempt identify the presence of apigenin and quercetin by HPTLC. The apigenin(5,7,4′ tri hydroxy flavone) and quercetin

were identified and quantified in our study. Apigenin was found in ethyl acetate extract of *T.grandis*(TGEAE) (0.7%) and Quercetin was found in ethanol extract of *T.grandis*(TGEE) (0.01%).**(Plate-21, Fig-8,9,Table-12,13)**

PHARMACOLOGICAL STUDIES:

Acute Toxicological study:

In continuation of our efforts to verify the safety of TGEAE we performed Brine shrimp lethality assay (BSLA) using free swimming hatched out *Artemianauplii* which based on the ability to kill laboratory cultured brine shrimp . It was observed that 100% of mortality above 7500ppm for TGEAE . LC_{50} for TGEAE were about 5000ppm and 10ppm respectively in 24hrs.(**Table-14**)

As a part of larger effect to develop anti-malarial agent we performed *P.falciparum* growth assay, a whole organism method allows all relevant blood stage target to be screened simultaneously using 96 well microtitre plates.[Fidock DA *et al.*,2004,Diallo A *et al.*,2008]

After the incubation the percentage of parasitemia in the control well has increased drastically after 96 hrs as compared to the parasite at 0 hrs. This increase shows the normal growth of the parasite in the RBCs . The percentage of increase of the parasite at 48 hrs, 72 hrs and 96 hrs have been tabulated.(**Table-15**)

The results were encouraging that is comparable to that of the standard drug chloroquine. The results was statistically significant($p<0.05$).

Therefore the above studies proved that the ethyl acetate extract of the leaves of *T.grandis* is therapeutically effective agent in malaria without toxicity and its effectiveness comparable to currently available therapeutic agent chloroquine.

In the standard well a 100% reduction was noticed during 48 hrs,72hrs and 96 hrs which indicates the action of the known anti-malarial drug chloroquine against the parasite. In

the test percentage of reduction of the parasite at 48 hrs is, 72 hrs is,96hrs is (**Plate-22).** This showed that ethyl acetate extract of *T.grandis* leaves induced a significant decrease *P.falciparum* proliferation. This shows it is an in expensive and affordable crude extract and can be evaluated further for formulations.From this investigation it is confirmed that *T .grandis*leaves is a cheap, easily accessible, safe and additive to conventional treatment of malaria.The activity may be due to the presence of polyphenols, betulinic acid, apigenin.[Aradhana R *et al.*,2010,Ghareeb MA *et al.*,2013]

The result provide scientific background for the leaves used as anti-malarial in folk medicine.

Limitations of study:

Some of the limitations include the use of organic extracts instead of aqueous extracts as prepared in the traditional setting. This means some of the compounds which may have a therapeutic effect in an aqueous extract may be absent in the test samples. This may have excluded antimalarial compounds that are polar resulting in lower antimalarial activity. Another limitation is the use of parasites at different growth stages. *Plasmodium* parasites were unsynchronized at the time of the experiments.This means that if an extract had an effect on a particular growth stage and not others, the antiplasmodial effect would be reduced. Additionally, a limited number of replicates of antiplasmodial activity assays were done. This may have introduced bias, as parasite growth patterns are variable depending on culture conditions; hence using more replicates would have resulted in more representative outcomes in the antiplasmodial assays. Finally, metabolic assays have to be carried out on only. Further information on the mechanism of action of the plant extracts could have been obtained using this *Plasmodium s*train.

It was reported that leaves of *T.grandis*used for wound healing in folk medicine. So we have planned to provide scientific background of this ethnomedical claims. So the restoration of damaged tissue as closely as possible to its normal state mainly studied. Our study confirms the *T.grandis*traditional claim for wound healing activity. Epidermal migration was measured (Nayak S, 2006).

Epidermal migration or keratinocyte migration distances from the edges of each wound were measured, normalized with the PBS control group and expressed as mean%. The result clearly showed TGEAE(3%) promoted statistically significant wound healing effect is comparable to the standard drug mupirocin.

Wound healing activity of ethyl acetate extract of *T.grandis* leaves may be due to its flavonoids, triterpenoids constituents. Both of them known to have astringent property which is responsible for wound contraction and increased rate of epithelialisation along with the supportive anti-microbial activity (Nayak S., 2006).

Here we want to emphasise the traditional use of the leaves for the treatment of diabetes and the several supportive scientific research of this claim as anti-diabetic and for wound healing activity[Manoharan KP *et al.*, 2007,Majumdar M *et.al.*,2007,Shukla N *et al.*,2010, Aradhana R *et al.*,2010, SajjadulkarimMd*et al.*,2011,Pradeep G *et al.*,2012, Bangou MJ *et al.*,2012, Khera N and Bhargava S.,2013, Yogesh Sharma *et al.*,2013].

The common complication of diabetic patient is wound as a adverse effect which are an enormous burden on the health care system, both in terms of cost and intensity of care required. Conclusively our study showed significant enhancement of wound repair and therefore can be beneficially, safely used as auxiallary therapy in diabetic patient with foot ulcers in addition to the other available treatment.

CHAPTER - 7

CONCLUSION AND RECOMMENDATION

The present investigation highlights the pharmacognostical, phytochemical and potential,(a new hope in the fight against apicomplexan*Plasmodiumfalciparum)* more effective antimalarial therapeutically comparable to the synthetic counterpart, without toxicity of the ethyl acetate extract of the leaves of *Tectonagranidis*Linn family Verbenaceae, a widely, easily available plant commonly called as Teak plant. Ethno medical information revealed that it was used in various ailments for long time all over the world. It is traditionally known to be useful for the treatment of wide panel of diseases like tuberculosis, various kinds of **wounds** especially burn wound, **malaria, anaemia**, leprosy, skin diseases like pruritis, stomatitis, indolent ulcers, haemorrhages, menstrual disorder, bone joint disease. Young leaves and fruits prescribed to women for fertility control and fumes of dry leaves to kill guinea worms. It also possesses anti- ulcer and anti- cancer activity. Various scientific investigations of the leaves showed anti-diabetic activity, anti-microbial activity, anti-oxidant, analgesic, anti-inflammatory, anti-amoebic, anti-hypertensive and wound healing activity.

So we have also investigated*ex vivo* wound healing activity to provide scientific grounds of its ethno medical claims and to support the previous reports. The tremendous economic potentiality of this cash crop remains neglected by the scientists, technologists, physician, traders, administrators, policy makers, farmers etc.

The morphological evaluation showed the adherence of general character to the family.

Detailed microscopical characters of the leaves showed the presence of usual leaf anatomical characters.T.S through midrib showed planoconvexdorsivenrtal leaf with slight raised in the centre hump, palisadecells, spongyparenchyma,few layesrs of collenchymas

below both the epidermis in thr midrib, vascular bundle, ground tissue with frequent calcium oxalate crystals,non glandular multicellular uniseriatetrichomes, apostomatic upper epidermis and lower epidermis with ranunculaceous stomata. Powder microscopy, microscopic schedules (Vein islet and termination numbers, stomatal number and index, palisade ratio), physicochemicalparameters(Ash values, extractive values, LOD) were evaluated and presented.

T.S of petiole showed more or less triangular with flat adaxial surface. The epidermis is covered with striated cuticle. Cortex contain outer collenchyma cells and inner polyhydral parenchyma cells. Vascular bundle contain xylem and phloem fibres. Central large pith region composed of parenchyma is present.

Scanning Electron Microscopy of midrib showed many folded appearance. No diagnostic feature and new kind of microcostituents not previously recognised and apparently simple structure which may be extremely complex was observed.

Preliminary phytochemical screening showed the presence of carbohydrates, proteins and amino acids, anthroquinone glycosides, flavonoids, saponin, terpenoids, tannins and phytosterols. Fixed oil, alkaloids, volatile oil were found to be absent.

Flavonoid content of TGEAE in terms of quercetin by aluminium chloride was found to be 13.5µg/g. Total phenolic content of TGEAE in terms of gallic acid was found to be 22.2µg/g. Trace elements analysis by energy dispersive X ray spectrometer (EDS) showed Mg (0.62), P (0.97), S (0.48),Cl (1.24), K(9.24),Ca (2.19% weight).The presence of apigeninwas identified and quantified (0.7%) by HPTLC which is reported to possesses antimalarial activity(quercetin 0.01% in TGEE) The **3R's** ethical principle (**R**eduction, **R**efinement,

and **R**eplacement) was implemented that help to minimize harms to vertebrate animals used in science.

Acute toxicity assessment using *Artemianauplii* bybrineshrimp lethality bioassay (BSLA)showed**LC_{50}** of TGEAE of the leaves 5000ppm& found non- toxic to the animal.

This study has been a contribution to the assessment of possible therapeutic properties of TGEAE of the leaves in an attempt to validate theirethnomedicinal uses in malaria. The presence of apigenin and polyphenols which have anti-malaria properties. Together with their antiplasmodial activities, it strongly supports theposition that the plant traditional usage is rational. Presence of lupine type betulinic acid may also be responsible for the activity. The antimalarial activity shown by this leaf also confirms the importance of using an ethnopharmacological approach in screening plants as a source for potential antimalarials. Its dual benefit is of scientifically proved antianaemic activity (Diallo A *et al.,* 2008) of another traditional use for anaemia which is a serious symptom in the malaria patient

The results were encouraging that it is comparable to that of the standard drug chloroquine. This research provide a pathway to a newer drug lead or herbal formulation

Traditionally claimed wound healing activity was evaluated by using *ex-vivo* porcine ear wound healing model (PEWHM). TGEAE of the leaves (3%) showed statistically significant wound healing effect which is comparable to the standard drug mupirocin which is the currently recommended drug for wounds and diabetic foot ulcer.

A previous reports confirm the antidiabetic activity of the leaves of T.grandis this drug may be useful in diabetic foot ulcer as an additional benefit[Shukle N *et al.,* 2010, Pradeep G *et al.,* 2012].

Further studies are needed for purification compounds in extract and to understand the complete mechanism of wound healing activity of *T.grandis*leavesIt may be suggested for treating various types of wounds in human beings. Development of wound healing agent from a natural drug is cheaper, safer and effective.

We recommend further investigations in animal model and clinical trials to confirm this potential therapeutic effect. These aspects remain to be studied.

Moreover the vast economic potentiality of this crop can be adequately exploited and can create employment opportunity to an agricultural worker throughout the year.

Recommendation:

In conclusion this study shows the value of traditional knowledge in the fight against apicomplexan parasite *Plasmodium.*It is possible to balance people's cultural believes and practices with the WHO objective of malaria elimination. The validation of plants used by the indigenous populations as described will facilitate in the ongoing efforts to eliminate malaria by providing treatment to the isolated rural populations that do not have access or to do not believe in conventional medicine and rely mostly on ethnomedicine.

Further *in vitro* investigations should include antimalarial screening and mode of action studies. Combination treatments based on use of plants with different mechanisms of action should be evaluated as well as repeat treatments with the extracts as these may be plasmodicidal and not just suppressive. *In vivo* studies in a small animal model of malaria should also be conducted to investigate how liver metabolism affects the efficacy of the plant treatments. These studies will also provide toxicological and pharmacological data to advise on dosage regimens. It is foreseen that the sum of these studies on scientific validation of the

medicinal uses of such plants may eventually contribute to the integration of ethno-medicine into mainstream malaria case management.

In addition our study showed significant enhancement of wound repair and therefore can be beneficially, safely used as auxiallary therapy in diabetic patient with foot ulcers in addition to the other available treatment along with its proved antidiabetic activity.

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