PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE LEAVES OF *Gmelina arborea Roxb.* (VERBENACEAE)

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University,

Chennai

In partial fulfillment of the requirements

For the award of the Degree of

MASTER OF PHARMACY
IN
PHARMACOGNOSY

SUBMITTED
BY
261220702

DEPARTMENT OF PHARMACOGNOSY
MADURAI MEDICAL COLLEGE
MADURAI – 625020

APRIL-2014

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Dated :

CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE LEAVES OF *Gmelina arborea Roxb.*(VERBENACEAE)''** submitted by **Miss. P.BALA (Reg. No.261220702)** in partial fulfilment of the requirement for the award of the degree of **MASTER OF PHARMACY** in **PHARMACOGNOSY** by The Tamil Nadu Dr. M.G.R. Medical University is a bonafide work done by her during the academic year 2013-2014 under the guidence of **Miss.R.GOWRI.M.Pharm.,Assistant Reader** in the Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai-625 020.

The dissertation is forwarded to the Controller of Examinations,The Tamil Nadu Dr.M.G.R. Medical University,Chennai.

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(Miss.R.GOWRI)

Project supervisor

- **Part B**

Protocol form for research proposals to be submitted to the committee / institutional animal ethics committee, for new experiments or extensions of ongoing experiments using animals other than non – human primates

Project title: **ANTI CONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF GMELINA ARBOREA AGAINST MAXIMAL ELECTRO SHOCK INDUCED CONVULSION IN RATS**

Chief Investigator

a. Name : Mr. N. Chidambaranathan, M.Pharm, Ph.D.,

b. Designation : Head, Department of Pharmacology,

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- List of names of all individuals authorized to conduct procedures under this proposal.
  A. Mrs. G. Nalini, M.Pharm., (Pharmacological Supervisor).

  B. Mr. N.Jegan.M.Pharm, (Pharmacological Supervisor).

- Funding source: Nil
- Duration of the project
  a. Number of months : Five months

  b. Date of initiation : 01.10.2013.

  c. Date of completion : 28-02-2014.

- If date by which approval is needed is less than six weeks from date of submission, justification for the same.

  NIL

- A] To evaluate the anti-inflammatory activity of **Ethanolic extract of gmelina arborea roxb.**
- Animals required:
  - Species: Albino Wistar rats.
  - Age/weight/size: 6 months, Rat (180-220gms) Medium.
  - Gender: Male and Female in equal ratios.
  - Numbers to be used: Total Numbers:24
  - Number of days each animal will be housed:30 Days.

(For IAEC / CPCSEA usage)

Proposal number	: P.Bala/M.Pharm/IAEC/121 /KMCP/ 261220702/2013-14
Date first received	:01.10.2013
Date received after modification (if any)	: NA
Date received after second modification (if any)	: NA
Approval date	: 10.10.2013
Expiry date	: 28.02.2014
Name of IAEC / CPCSEA chairperson	: N.CHIDAMBARANATHAN

Date:10.10.2013

CPCSEA NOMINEE
INSTITUTIONAL ANIMAL ETHICS COMMITTEE
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MADURAI-625 107

Signature

I. A. E. C. CHAIRMAN
INSTITUTIONAL ANIMAL ETHICAL COMMITTEE
K. M. COLLEGE OF PHARMACY
MADURAI-625 107.

ANNEXURE

Investigator declaration

- I certify that I have determined that the research proposal herein is not unnecessarily duplicate of previously reported research.
- I certify that all individuals working on this proposal and experimenting on the animals have been trained in animal handling procedures.
- For procedures listed under item 11, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.
- I will obtain approval from the IAEC / CPCSEA before initiating any significant changes in this study.
- Certified that performance of experiment will be initiated only up on review and approval of scientific intent by appropriate expert body (institutional scientific advisory committee / funding agency / other body (to be named)
- Institutional biosafety committee (IBC) certification of review and concurrence will be taken (required for studies utilizing DNA agents of human pathogens)
- I shall maintain all the records as per format (Form D)

Signature

(P.Bala)

Name of Investigator

I. A. E. C. CHAIRMAN
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Dr. (Mr.) D. STEPHEN., M.Sc., Ph.D.,

ASSISTANT PROFESSOR,

DEPARTMENT OF BOTANY,

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MADURAI - 625002.

CERTIFICATE

This is to certify that the specimen brought by **Miss. P.BALA** ,II.M. Pharmacy, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai is identified as ***Gmelina arborea Roxb.*** belonging to the family **Verbenaceae.**

SSTATION : Madurai.

DATE :

(Dr. D. STEPHEN)

Dr. D. STEPHEN, Ph.D.,
LECTURER IN BOTANY
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P.BALA

CONTENTS

INTRODUCTION

CHAPTER-I

INTRODUCTION

The Indian subcontinent is enriched by a variety of flora- both aromatic and medicinal plants. This is due to the wide diversity of climatic conditions in India ranging from deserts to swam plants. In India, 75% of the populations depend on the traditional system of medicines for relief. In the early stages, the science of medicine developed around those plants which had curative properties. A continued search for medicinal plants during the last several centuries has given rise to a long list of plants which are of great use in the treatment of diseases and for promoting health.

The WHO is now actively encouraging developing countries to use herbal medicine with they have been traditionally used for centuries. Natural products as a basis for new drugs have great promise and it is gratifying to note that the WHO have shown an abiding interest in plant derived medicines, described in the folklore of various countries.[1]

Traditional remedies invariably involve crude plant extracts containing multiple chemical constituents, which vary in potency from highly active to very weak. [2]

WORLD SITUATION ON TRADITIONAL MEDICINE [3]

Traditional medicine is a very important part of health care. Most population in the developing countries still relies mainly on indigenous traditional medicine for satisfying their primary health care needs. Traditional medicine has not however been incorporated in most national health systems and the potential of services provided by traditional practitioners is far from being fully utilized. Herbal medicines are of great importance to the health individuals and communities but their quality assurance needs to be developed. During the last decade, in many developed countries there has also been a growing interest in herbal

medicine, acupuncture and alternative system of medicines and other types of traditional medicines has occurred. Proper use of these different types of medicine has therefore become a concern.

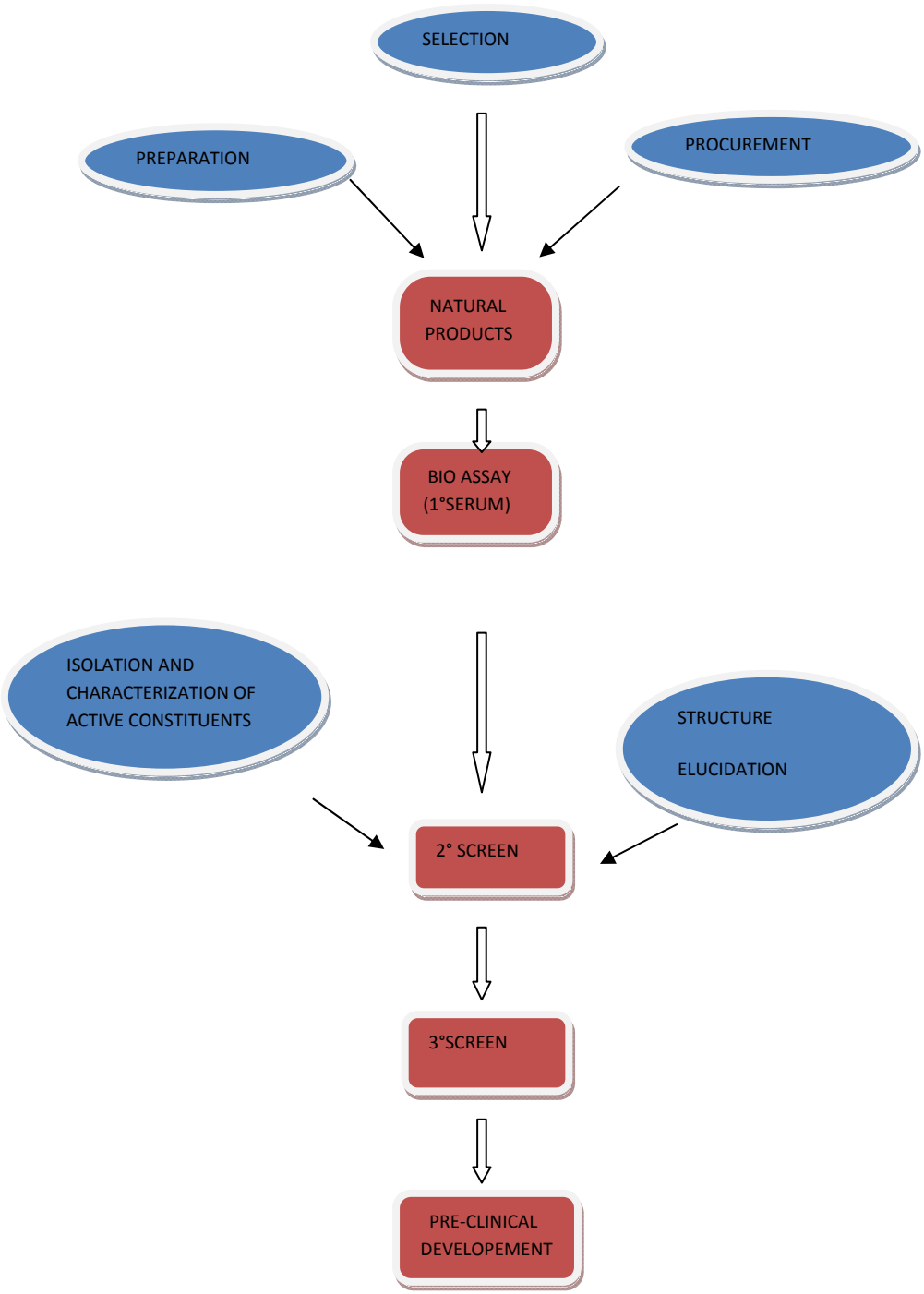
HERBAL DRUGS IN TRADITIONAL MEDICINES

Herbal drugs constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive.

DRUG DISCOVERY FROM NATURAL PRODUCTS

According to surveys and other research carried out in different countries, scientific medicine in developing countries serves only a minority, while the rest of the population attends to its health needs through the process called traditional medicine, aboriginal medicine, or folk medicine, processes based essentially on the use of low-cost medicinal plants that are easily accessible to the entire population.

Various strategies for the discovery of drugs from natural resources:



About 30% of the drugs sold worldwide are based on natural products. Though recombinant proteins and peptides account for an increasing market volume, the superiority of low- molecular- mass compounds in human disease therapy remains undisputed, mainly due to more favorable compliance and bioavailability properties. In the past, new therapeutic approaches have often evolved from research involving natural products. Numerous examples from medicine impressively demonstrate the innovative potential of natural compounds and their impact on progress in drug discovery and development. [4]

EPILEPSY

Epilepsy is a common neurological abnormality, affecting about 1% of world population. A seizure means paroxysmal abnormal discharge at high frequency from aggregate of neurons in cerebral cortex. Epilepsy is a condition characterized by recurrent episodes of such seizures. Inspite of several advancements in the field of synthetic drug chemistry, plants continue to be one of the major raw materials for drugs treating various ailments of humans. Plant remedies are effective and without side effects provided recovery, if they are selected properly [5-6].

The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy [7-9]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [10]. Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity [11] and many such plants are yet to be scientifically investigated.

Eg:

- Ricinous communis L
- Datura stamoniumL
- Bacopa monnieri(L)
- Allium sativum L
- Asparagus racemosus willd.

For the purposes of drug treatment, it is more useful to classify patients according to the type of seizure they experience.

Nature and mechanism of seizures

Almost a century ago, John Hughlings Jackson, the Father of modern concepts of epilepsy, proposed that seizures were caused by "occasional, sudden, excessive, rapid and local discharges of gray matter", and that a generalized convulsion resulted when normal brain tissue was invaded by the seizure activity initiated in the abnormal focus. The electroencephalogram (EEG) amply demonstrates that seizures are associated with abnormal and sometimes massive electrical discharge in the brain and serves as the basic method of differential diagnosis of the epilepsies.[12]

Classification of epilepsies

I. Generalized seizures

a) Generalized tonic-clonic seizures
b) Absence seizures
c) Atonic seizures
d) Myoclonic seizures
e) Infantile spasms (Hypsarrhythmia)

II. Partial seizures

a) Simple partial seizures
b) Complex partial seizures
c) Simple partial or complex partial seizures secondarily generalized.[13]

Medication for epilepsy

Epilepsy cannot be cured with medication. However, with the right type and strength of medication, the majority of people with epilepsy do not have seizures. The medicines work by stabilizing the electrical activity of the brain. We need to take medication every day to prevent seizures.

Medicines used to treat epilepsy include: carbamazepine, sodium valproate, lamotrigine, phenytoin, oxcarbazepine, ethosuximide, gabapentin, levetiracetam, tiagabine, topiramate, vigabatrin, phenobarbital, primidone and clonazepam. They come in different brand names.

Other medication

- **Surgery** to remove a small part of the brain which is the underlying cause of the epilepsy. Surgery is only possible for a minority of people with epilepsy and it may be considered when medication fails to prevent seizures, especially partial seizures. Only a small number of people with epilepsy are suitable for surgery and, even for those, there are no guarantees of success. Also, there are risks from operations. However, surgical techniques continue to improve and surgery may become an option for more and more people in the future.
- **Vagal nerve stimulation** is a treatment for epilepsy where a small generator is implanted under the skin below the left collar bone. The vagus nerve is stimulated to

reduce the frequency and intensity of seizures. This can be suitable for some people with seizures that are difficult to control with medication.

- **The ketogenic diet** is a diet very high in fat, low in protein, and almost carbohydrate-free. This can be effective in the treatment of difficult-to-control seizures in some children.
- **Complementary therapies** such as aromatherapy may help with relaxation and relieve stress, but have no proven effect on preventing seizures.
- **Counseling**. Some people with epilepsy become anxious or depressed about their condition. A doctor may be able to arrange counseling with the aim of overcoming such feelings. Genetic counseling may be appropriate if the type of epilepsy is though to have an hereditary pattern.[14]

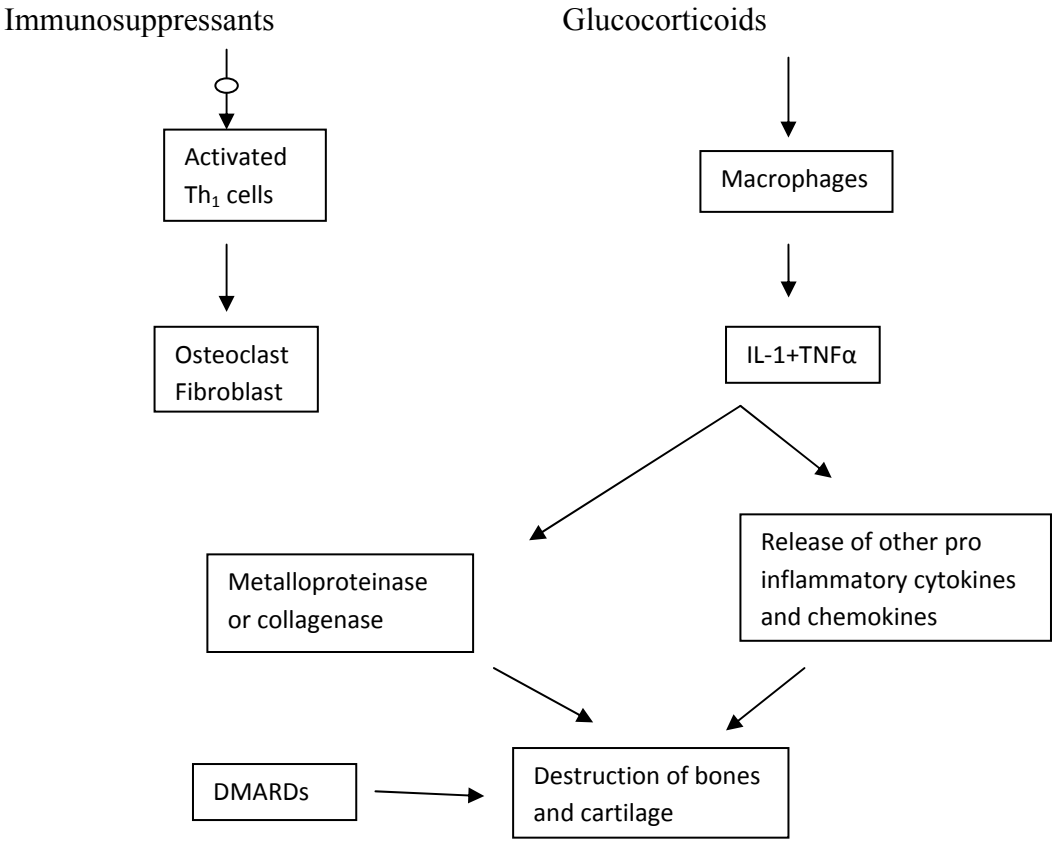
Mechanism of action of anti-epileptic drugs

a) Enhancement of GABA action
b) Inhibition of sodium channel function
c) Inhibition of calcium channel function[15]

RHEUMATOID ARTHRITIS [16]

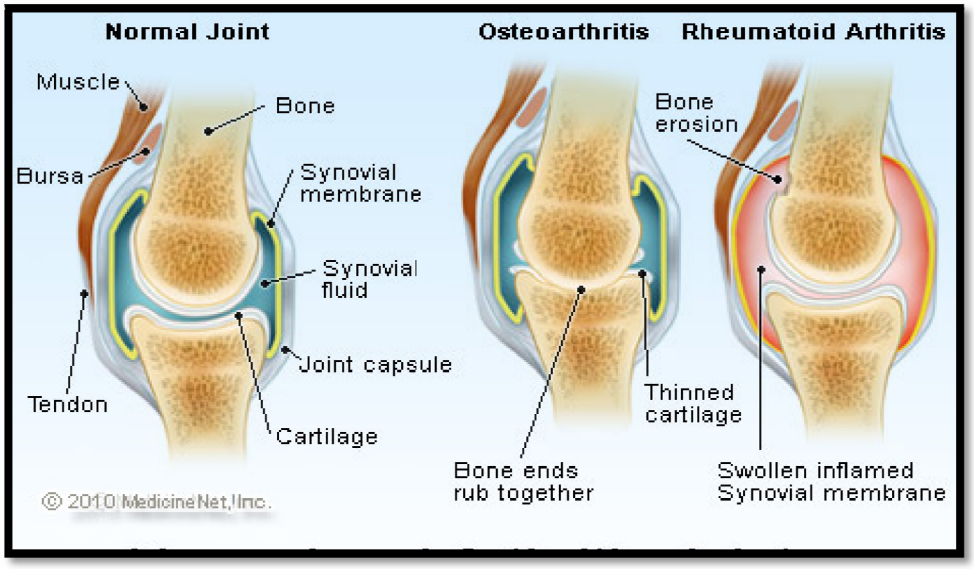
Rheumatoid arthritis is an immunologic disease that not only affects mobility but the quality of life as well. NSAIDs provide only symptomatic relief in inflammation and pain but have little effect on the progression of bone and cartilage destruction where cytokines IL-1 and TNFα play a major role in pathogenesis.

PATHOGENESIS OF RHEUMATOID JOINT DAMAGE AND SITES OF ACTION OF ANTIRHEUMATOID DRUGS



Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints. While inflammation of the tissue around the joints and inflammatory arthritis are characteristic features of rheumatoid arthritis, the disease can also cause inflammation and injury in other organs of the body. Autoimmune diseases are illnesses that occur when the body's tissues are mistakenly attacked by their own immune system. The immune system contains a complex organization of cells and antibodies designed normally to "seek and destroy" invaders of the body, particularly infections. Patients with autoimmune diseases have antibodies in their blood that target their own body tissues, where they can be associated with inflammation. Because it can affect multiple other organs of the body, rheumatoid arthritis is referred to as a systemic illness and is sometimes called rheumatoid disease.

FIG.1: NORMAL AND ARTHRITIC JOINTS



Rheumatoid arthritis is a chronic illness, meaning it can last for years, patients may experience long periods without symptoms. However, rheumatoid arthritis is typically a progressive illness that has the potential to cause joint destruction and functional disability.

A joint is where two bones meet to allow movement of body parts. Arthritis means joint inflammation. The joint inflammation of rheumatoid arthritis causes swelling, pain, stiffness, and redness in the joints. The inflammation of rheumatoid disease can also occur in tissues around the joints, such as the tendons, ligaments, and muscles.

In some people with rheumatoid arthritis, chronic inflammation leads to the destruction of the cartilage, bone, and ligaments, causing deformity of the joints. Damage to the joints can occur early in the disease and be progressive. Moreover, studies have shown that the progressive damage to the joint does not necessarily correlate with the degree of pain, stiffness, or swelling present in the joints.

Rheumatoid arthritis is a common rheumatic disease, affecting approximately 1.3 million people in the United States, according to current census data. The disease is three times more common in women as in men. It afflicts people of all races equally. The disease can begin at any age and even affects children (juvenile rheumatoid arthritis), but it most often starts after 40 years of age and before 60 years of age. In some families, multiple members can be affected, suggesting a genetic basis for the disorder [17]

Symptoms and diagnosis of rheumatoid arthritis

Symptoms include joint swelling, pain, morning joint stiffness, poor sleep, fatigue, loss of weight and feeling of having flu symptoms. Rheumatoid arthritis is diagnosed by rheumatoid factor these are abnormal antibodies (IgG) which are present in blood. These are reacted with antigen and form antigen-antibody complex that leads to pain and inflammation of synovial membrane. The American College of Rheumatology requires at least four of the following seven criteria to confirm the diagnosis [18-19]

• Morning stiffness around the joint that lasts at least 1 hour

• Arthritis of three or more joints for at least 6 weeks

• Arthritis of hand joints for at least 6 weeks

• Arthritis on both sides of the body for at least 6 weeks

• Rheumatoid nodules under the skin

• Rheumatoid factor present in blood testing

• Evidence of rheumatoid arthritis on X-rays

Principles of management of rheumatic arthritis [20]

- Rest to acutely inflamed joints
- Reduction of pain and inflammation by use of anti inflammatory drugs.
- Prevention of articular damage by early use of drugs modifying the disease course (DMARD) with rapid escalation.
- Use of combination therapy.
- Use of glucocorticoids as ancillary measures.
- Graded exercises and physiotherapy.
- Treatment of comorbid conditions such as infection and osteoporosis.
- Use of biological agents if DMARD fails.
- Modification of the diet using more omega-3 fatty acids in place of omega-9 fatty acids in their respect, vegetarian diet may be beneficial.

REVIEW OF LITERATURE

CHAPTER - II

REVIEW OF LITERATURE

The literature review of *Gmelina arborea (Roxb.)* reveals that the following works have been already done on the plant.

PHARMACOLOGICAL ACTIVITY

1. **Shirwaikaret.A *et al*., (2003)** have reported the alcoholic extract of leaves of *Gmelina arborea* at the dose level of 200mg/kg increased the wound contraction rate, skin breaking strength, hydroxyl proline content and dry granuloma weight and decreased epithelization period in the testominals.[21]

2. **Panja.A.K. *et al*., (2003)** have reported the role of Dasamool in controlling hypothyroidism .Dasamool consist of 11 plants like *Gmelina arborea,* Oroxylumindicum etc. [22]

3. **Sangeetasinha *et al*., (2006)** have reported the effect of aqueous extract of *Gmelina arborea* bark and fruit on paraquat and H_2O_2 induced oxidative stress using liver slice culture. Both paraquat & H_2O_2 were found to be cytotoxic as measured by release of lactate dehydrogenase from liver slice culture. Addition of bark & fruit extracts along with these cytotoxic agents led to decrease in lactate dehydrogenase release. [23]

4. **Patil.S.M. *et al*., (2009)** have studied the antioxidant activity of methanolic extracts of stem bark of *Gmelina arborea Roxb* using various in-vitro assays. The antioxidant activity of MEGA was evaluated by using DPPH method, reducing power assay, nitric oxide scavenging activity &H_2O_2 scavenging activity. Total phenolic content was also determined by using gallic acid as a standard. [24]

5. **Kulkarn.Y *et al*., (2010)** have reported the aqueous extract of *Gmelina arborea* was found safe in acute toxicity studies when tested in rodents. [25]

6. **Nayak *et al*., (2012)** have reported the antibacterial, antioxidant &anti diabetic activities of different extracts of fruits of plant *Gmelina arborea* using ethanol, ethyl acetate, n-butanol &petroleum ether as solvents. The ethanol extract showed good antioxidant activity when compared to other three extracts. All the extracts were able to reduce sugar level in blood. Ethanol extract was found to have good antidiabetic activity in comparison to other extracts. **[26]**

7. **Bhabani Shankar Nayak *et al*., (2012)** have reported the diuretic effect of different fruit extracts of the plant *Gmelina arborea* using ethanol, ethyl acetate, n-butanol &petroleum ether as solvents. The n-butanol extract showed better diuretic effect in comparison to other extracts. **[27]**

PHYTOCHEMICAL STUDIES

8**. Hosny .M *et al*., (1998)** have isolated 12 new acylated iridoid glycosides named gmelinosides A-L (2-13) from the leaves of *Gmelina arborea*. **[28]**

9. **Sharma. A *et al*., (1998)** have reported *that the Gmelina arborea* was highly salt sensitive and withstood salinity upto 2.5 ds/m. **[29]**

10. **Thirunavoukkarasu.M *et al*., (1998)** have studied the micropropagation of *Gmelina arborea* through axillary bud culture and reported that the epicromic &crown regions of a 6 year old *Gmelina arborea* tree were used to initiate shoot cultures. Explants from the epicromic region produced 2.7+0.1 shoot less/explants when cultured on MC cown's medium for woody plants supplemented with 2% sucrose. About 80% of the in-vitro regenerated shoots rooted on IBA (1.5mg/l) supplemented MC cown's medium within 7-9 days of culture. **[30]**

11. **VinayaS.Ghate *et al*., (1998)** have evaluated Bruhatpanchmula germplasm. Stem cuttings of *Gmelina arborea* shows 6% rooting response using quick dip method in IBA (4000ppm).vegetative propagation was successful in *Gmelina arborea.* **[31]**

12. **Behera .P.R *et al*., (2008)** have regenerated *Gmelina arborea* using cotyledonary node explants. Multiple shoots were induced on Murashige &Skoog's medium supplemented with 6-BA, TDZ. [32]

13. **Moronkola D.O *et al*., (2009)** have reported the chemical composition of the fruit oil of *Gmelina arborea* by capillary gas chromatography, gas chromatography and mass spectrometry. [33]

14**. ShafaqS.S. *et al*., (2011)** have analysed the aqueous & hydro alcoholic extracts of stem bark of *Gmelina arborea* by HPTLC using umbelliferone. The percentage of umbelliferone in both extracts was 0.091%w/w & 0.069%w/w.[34]

ETHNOMEDICAL INFORMATION

15. **U.P.Bhattachary *et al*., (1998)** have reported the uses of *Gmelina arborea* for treating leach-bite, snake bite, scorpion-sting, rat bite and insect bite. [35]

16. **Devi Y.N *et al*., (2008)** have studied the ethnobotany survey for the family verbenaceae. *Gmelina arborea* has immense food and medicinal value.[36]

OTHER SPECIES

Gmelina asiatica

PHARMACOLOGICAL ACTIVITY

1. **Nallellasriramulu *et al*., (1998)** have reported the *Gmelina asiatica* was used in veterinary practices.[37]

2.**Madhu K.B *et al*.,(2001)** have studied the hexane & methanolic extract of *Gmelina asiatica* leaves for antimicrobial activity against 6 gram-positive bacteria,4 gram- negative

bacteria ,3 fungi & 3 yeast by the cup plate agar diffusion method. Methanolic extract showed very good antimicrobial activity against all the selected strains .Hexane extracts were devoid of activity. [38]

3. **Kottaimuthu .R. *et al*., (2009)** have reported that the *Gmelina asiatica* stem was used for leucorrhoea. [39]

4. **MadhuKatyayani *et al*., (2010)** have reported the presence of lignans in *Gmelina asiatica* roots. The study evaluated the effect of ethyl acetate extract of roots (EGAR) on estrogen receptor positive (MCF-T) and negative (MDA-MB-231) human breast cancer cell lines. The IC_{50} of EGAR on the cells was determined using MTT assay kit. [40]

PHYTOCHEMICAL STUDIES

5. Satyanarayana T. *et al*., (2007) have studied the phytochemical nature of *Gmelina asiatica* root. The bioguided extraction and fractionation of the alcoholic extract of the roots of *Gmelina asiatica* afforded a new flavone derivatives named oratifolin which has been isolated & identified together with the known compounds (+)sesamin, sakuranetin, (-) piperitol,(+) pinoresinol**.** [41]

ETHNOMEDICAL INFORMATION

6. **VenkataRaju RR *et al*., (1998)** have reported the ethnomedical information like vernacular names, medicinal property, part used &mode of administration for *Gmelina asiatica*. [42]

AIM AND SCOPE

CHAPTER-III

AIM AND SCOPE OF THE STUDY

Gmelina arborea is an unarmed, moderately sized to large deciduous tree, about 30 m or more in height and a diameter of upto 4.5 m. [43] Bark and the root are of medicinal value and used by Santhal and Gondbheels. The fruit juice is demulcent and used in curing gonorrhoea and cough and also to remove foetid discharges and worms from the ulcer. [44]

The present study has been planned to carry out the pharmacognostic, phytochemical and pharmacological evaluation of the ethanolic extract of the leaves of *Gmelina arborea.*

1. **Pharmacognostical studies**

- Macroscopical studies.
- Microscopical studies
- Quantitative analytical parameters
- Physico chemical parameters
- Powder analysis

2. Phytochemical studies

- Organoleptic evaluation
- Preliminary phytochemical screening
- Quantitative estimation of phytoconstituents
- Chromatography (TLC & HPTLC)

3. Pharmacological studies

A. In vitro antioxidant activity

- DPPH Assay
- Reducing power assay
- Phosphomolybdenum method

B. In-vitro anti-arthritic activity

C. In-vivo anti -epileptic activity

PLANT PROFILE

CHAPTER-IV

PLANT PROFILE

Gmelina arborea is a beautiful fast growing deciduous tree occurring naturally throughout greater part of India. The genus was named after J.C.Gmelin, an 18thcentury German botanist. The species name means treelike, from the Latin 'arbor' (tree) [45]

BIOLOGICAL SOURCE : *Gmelina arborea Roxb.*

FAMILY : Verbanaceae.

SYSTEMATIC POSITION

Kingdom	: Planta
Subkingdom	: Angiosperm
Super division	: Eudicots
Division	: Asterids
Class	: Equisetopsida
Order	: Laiales
Family	: Lamiaceae
Genus	: Gmelina
Species	: G.arborea

SYNONYM

Gmelina rheedii Hook.

COMMON NAME

Umi – thekku

VERNACULAR NAME

English	: Candhar tree, White teak.
Sanskrit	: Kasmari
Hindi	: Gambhar, Khambhari

Kannada : Shivanigida, Shivani

Malayalam : Kumizhu, Kumpil

Telugu : Pegummudu, Pegusmmadi, ummithekku

Tamil : Kumishan, Kumizhan

GEOGRAPHICAL DISTRIBUTION

Throughout India, Ceylon-Malayan and Philippine Islands. In India, *Gmelina arborea* occurs extensively from the Ravi eastwards in the sub-Himalayan tracts, common throughout Assam and adjoining areas of Northern West Bengal ,also in southern Bihar and Orissa.

HABIT AND HABITAT OF THE PLANT

It is a moderate sized unarmed deciduous tree, reaching 30m high and occurs in a variety of forest habitats. Bark grayish yellow, rather corky; branchlets and young parts clothed with fine white mealy pubescence. *Gmelina arborea* is a fast growing tree, which grows on different localities and prefers moist fertile valleys with 750–4500 mm rainfall. It does not thrive on ill-drained soils and remains stunted on dry, sandy or poor soils; drought also reduces it to a shrubby form.

ETHNOMEDICAL INFORMATION

1. The leaf paste is used for cephalgia and the leaf juice is a good wash for foul ulcers.

2. The flowers are used in treating leprosy and skin diseases.

3. The fruits are used for promoting the growth of hair and for anaemia, leprosy, ulcers, constipation, strangury, leucorrhoea and colitis.

4. The root is used for hallucination, fever, dyspepsia, hyperdipsia, haemorrhoids.Bark is useful in fever and dyspepsia.[45-48]

PHARMACOGNOSTICAL STUDIES

Fig.2: HERBARIUM OF *Gmelina arborea Roxb*.

CHAPTER-V

PHARMACOGNOSTICAL STUDIES

Collection of specimen

The leaves of *Gmelina arborea Roxb*. was collected in and around Madurai Medical College Campus during the month of August. The leaves were authenticated by a taxonomist at American College, Madurai. A voucher specimen of the leaves of *Gmelina arborea Roxb.* was placed at the Dept.of Pharmacognosy, College of Pharmacy, Madurai Medical College. A copy of the same was presented in **Fig.2.** The fresh leaves were used for the macroscopic, microscopic evaluation and for the determination of leaf constants. The leaves were collected, washed and shade dried. The shade dried leaves were powdered, sieved and used for physico chemical analysis and for powder microscopy.

SECTION-A

MACROSCOPICAL STUDIES

Gmelina arborea is a large deciduous tree with a straight trunk. It is wide spreading with numerous branches forming a large shady crown [49, 50]

Bark

The tree has smooth, pale ashy- grey or grey to yellow bark with black patches and conspicuous corky circular lenticels.

Leaves (Fig.4.1, 4.2)

Leaves are simple, opposite- decussate, broadly ovate, 10-25cm long and 7-20cm wide, apically long acuminate apex or caudate, entire on mature plants but strongly toothed or lobed on young plants, cordate or trunkate base, densely tomentose above when young, becoming glabrous above when mature, permanently densely fulvulous-tomentellous with

Fig.3: HABIT & HABITAT OF *Gmelina arborea*

Fig.4.1: DORSAL VIEW OF THE LEAF OF *Gmelina arborea*

Fig.4.2: VENTRAL VIEW OF THE LEAF OF *Gmelina arborea*

Fig.5: FLOWERS OF *Gmelina arborea*

Fig.6: FRUIT OF *Gmelina arborea*

stellate hairs beneath and shortly cuneate; petioles cylindrical 5-15cm long, puberulous or glabrous and glandular at the top.

Flowers (Fig.5)

Flowers appears with or sometimes before the young leaves, usually in small cymes of about 3 flowers on the densely fulvous-hairy panicle branches reaching 8 to 40cm long. Flowers are abundant, scented, reddish, brown or yellow and about 2.5 to 5cm in diameter; bracts 8mmlong,linear-lanceolate; calyx broadly campanulate,densely fulvous-hairy externally, the rim with 5small,triangular, acute teeth; Corolla large, showy varying from brilliant orange to reddish or brownish yellow, tubular below, obliquely funnel form at the throat, densely hairy outside, reaching 3.8cm long, the limb 2-lipped,the upper lip often orange, deeply divided into 2 oblong ,obtuse, backwardly curled lobules, the lower lip often lemon yellow, upto twice as long as the upper and 3-lobed.

Fruits (Fig.6)

Drupe, 2-2.5cm long, obovoid or pyriform, seated on the enlarged calyx, glossy and yellow when ripe; exocarp succulent and aromatic; endocarp bony and usually
2- celled. Seeds 1-3, lenticular and exalbuminous.

Root bark

The mature root-bark when fresh is yellow in colour. Dry pieces are curved and channeled, thinner ones forming single quills. The external surface is ryesed due to the presence of vertical cracks, ridges, fissures and numerous lenticels.

SECTION –B

MICROSCOPICAL STUDIES

Materials and methods [51-62]

The leaves of the plant were subjected to microscopical evaluation. The samples of leaves were cut and removed from the plant and fixed in FAA (Formalin, 5ml; Acetic acid, 5ml; Ethyl alcohol, 90ml). After 24h of fixing, the specimens were dehydrated with graded series of t-butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (M.pt-58-60^{o}C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with help of rotary microtome. The thickness of the sections was 10-12µm. De-waxing of the sections was carried out by customary procedure. The sections were stained with toluidine blue, since it is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also occurred. The dye rendered pink color to the cellulose walls, blue to the lignified cells, violet to the mucilage and blue to the protein bodies and also stained with safranin.

Leaf clearing

Paraffin embedded leaf was used for para-dermal sections. From these sections, the epidermal layers as well as vein islets were studied. Another method employed was clearing leaf fragments by immersing the material in alcohol (to remove chlorophyll) followed by treating with 5% sodium hydroxide. The material was rendered transparent due to loss of cell

contents. Epidermal peeling by partial maceration employing Jeffrey's maceration was also done. Glycerin mounted temporary preparations were made for cleared materials.

Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell component were studied.

Photomicrographs

The photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations bright field was used and for the study of starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized they appear bright against dark background. The magnifications of the anatomical features are indicated by the scale-bars in the photographs.

Microscopical observation

Leaf

The leaf consists of a thick midrib and a thin lamina with densely tomentose abaxial surface (**Fig.7.1**).The midrib is flat on the adaxial side and thick and wide on the abaxial side. (**Fig.7.2**) The lateral vein is also prominent with thick, hanging abaxial part (**Fig.7.3**).

The midrib is 550µm thick and 520µm wide. The epidermal layer consists of small, papillate thick walled cells. The ground tissue is homogenous and includes thin walled, compact parenchyma cells. The vascular strand consists of two arc shaped collateral bundles. There are short parallel lines of small thick walled xylem elements and thick arc of phloem situated on the lower part of the xylem strand. (**Fig.8.1**)

The lateral vein also has small, slightly papillate thick walled epidermal cells, homogenous compact parenchyma cells and single bowl shaped collateral vascular bundle (**Fig.7.3**)

Fig.7.1:T.S OF LEAF THROUGH MIDRIB

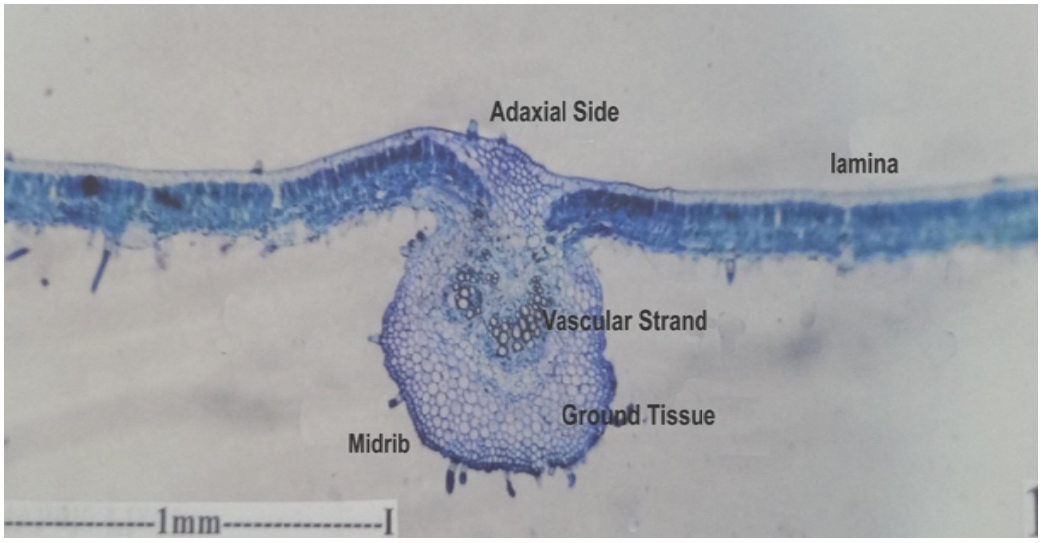


Fig.7.2: TS OF MIDRIB ENLARGED

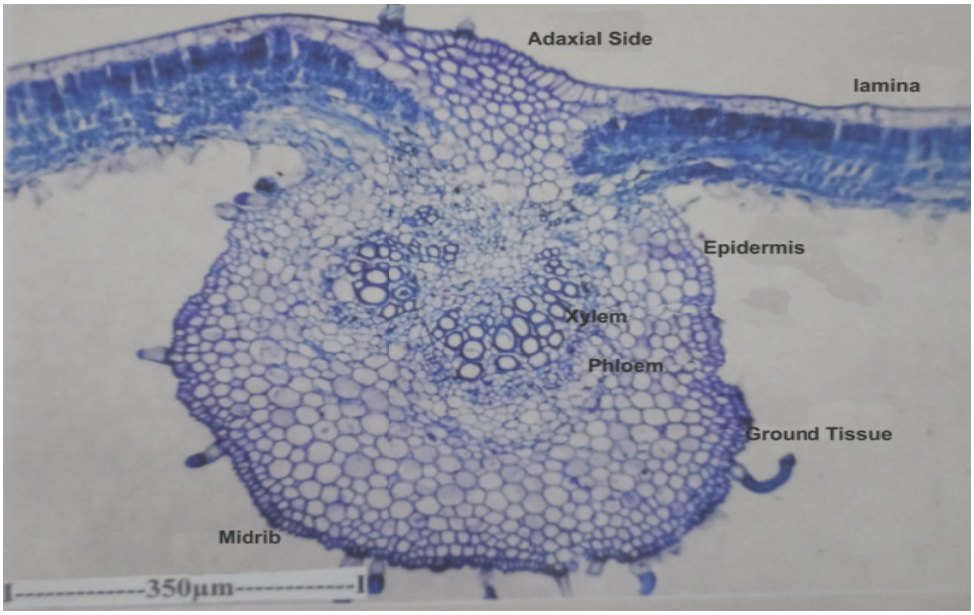


Fig.7.3: TS OF LATERAL VEIN

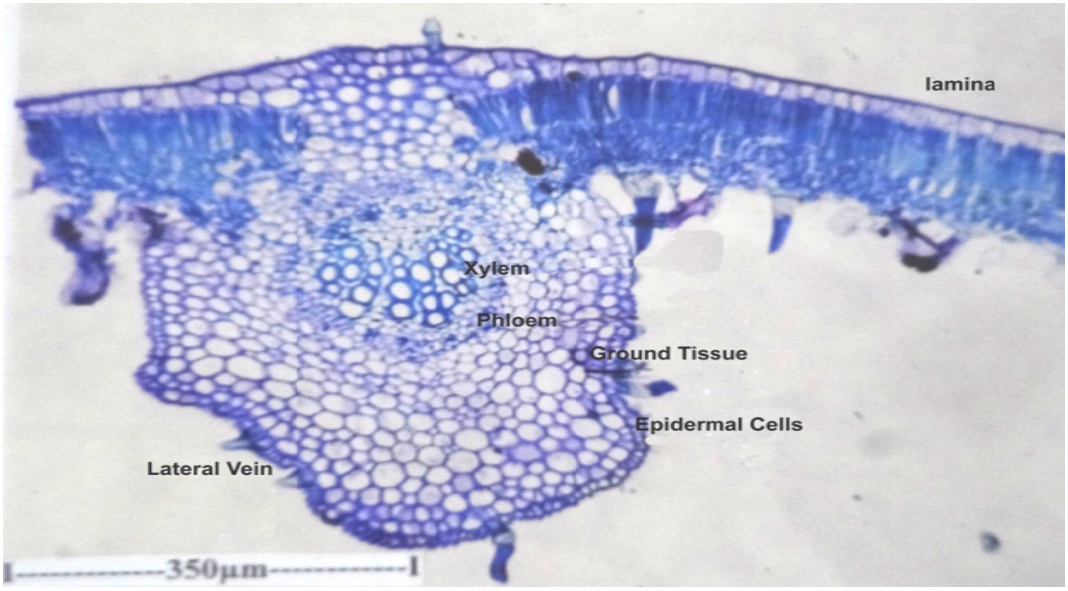


Fig.8.1:TS OF LAMINA

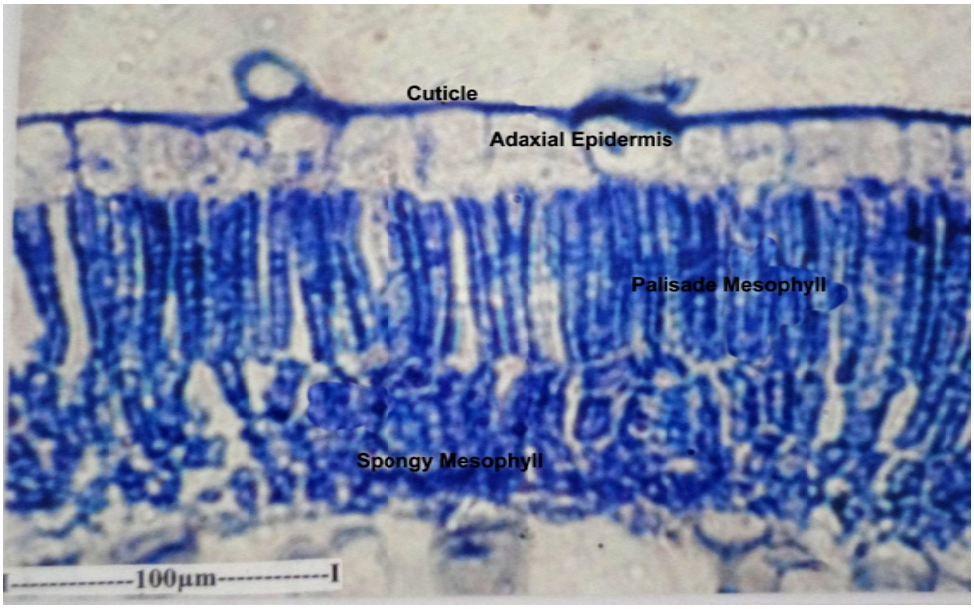


Fig.8.2:GLNDULAR TRICHOMES

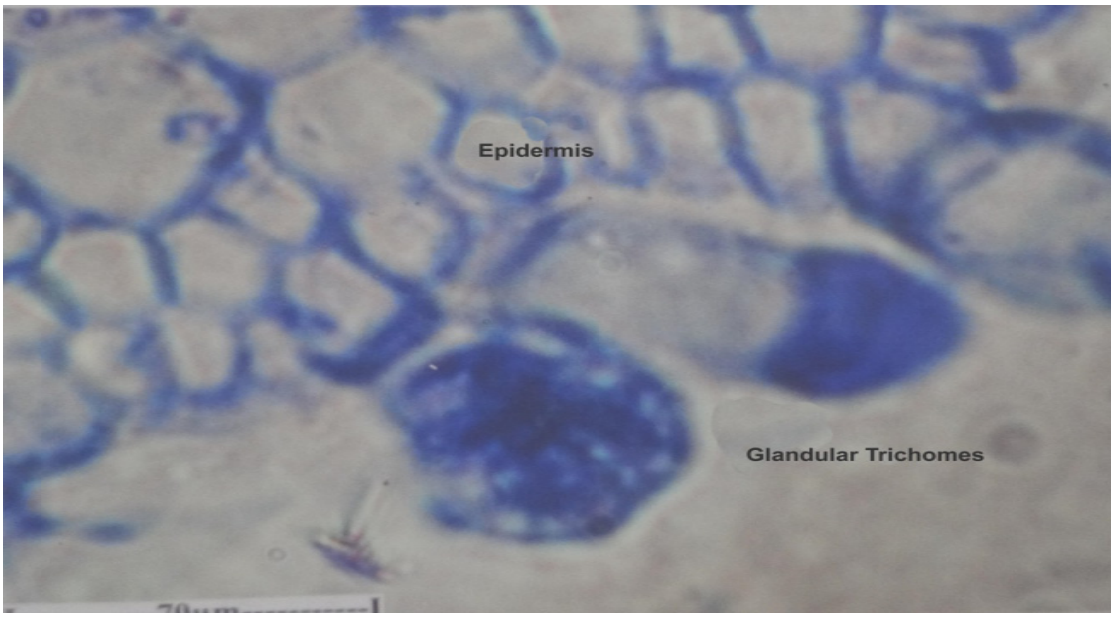


Fig.9.1: VENTRAL PATTERN OF THE LAMINA

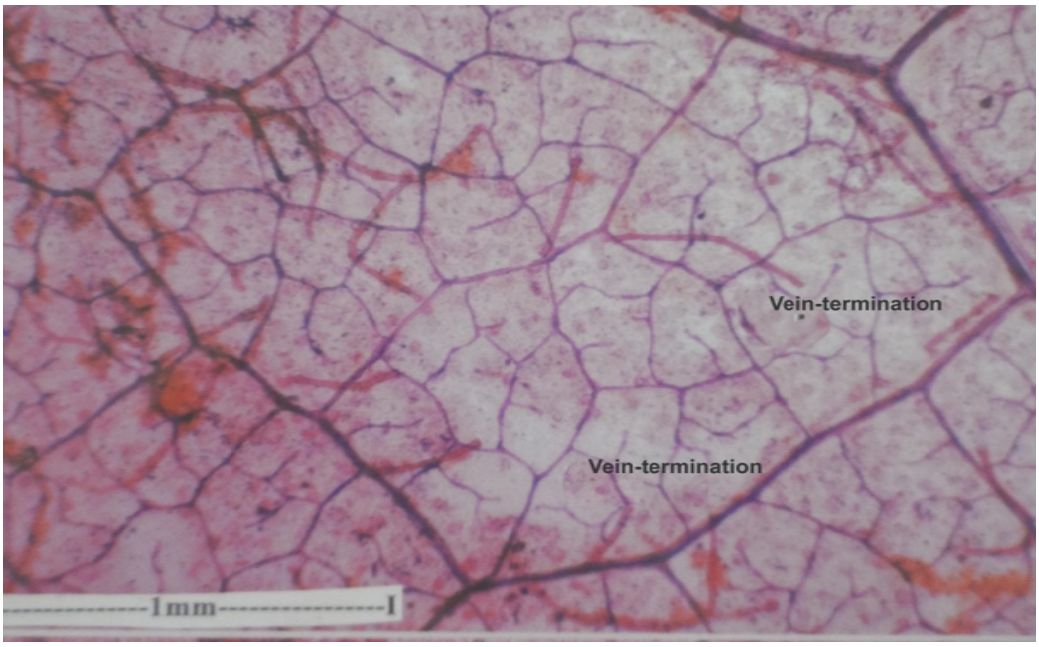


Fig.9.2:VEIN-ISLETS (VI) AND VEIN-TERMINATIONS (VT)

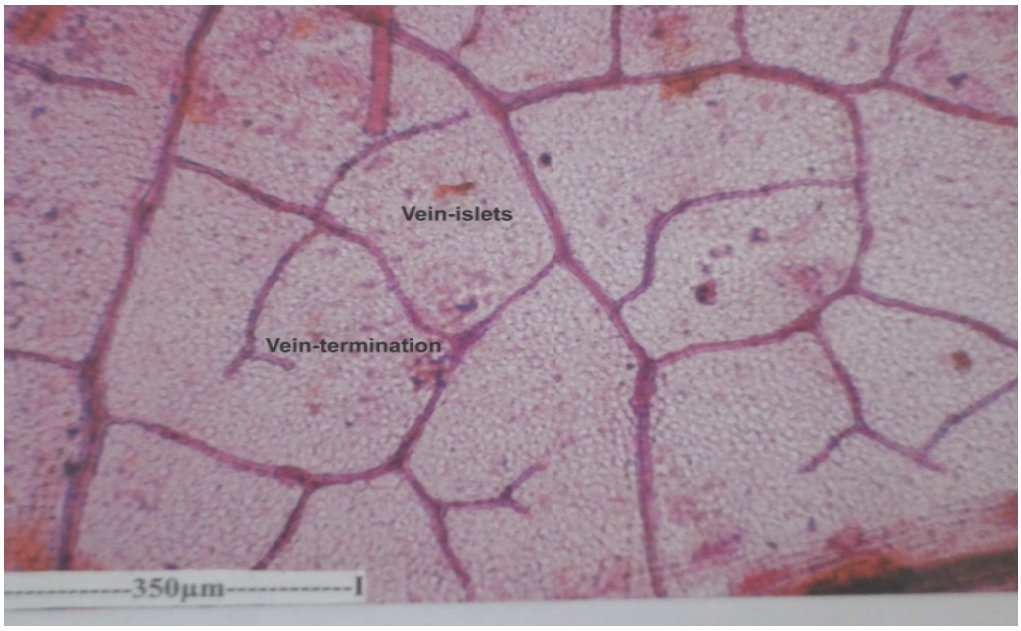


Fig.10.1:EPIDERMAL TRICHOMES

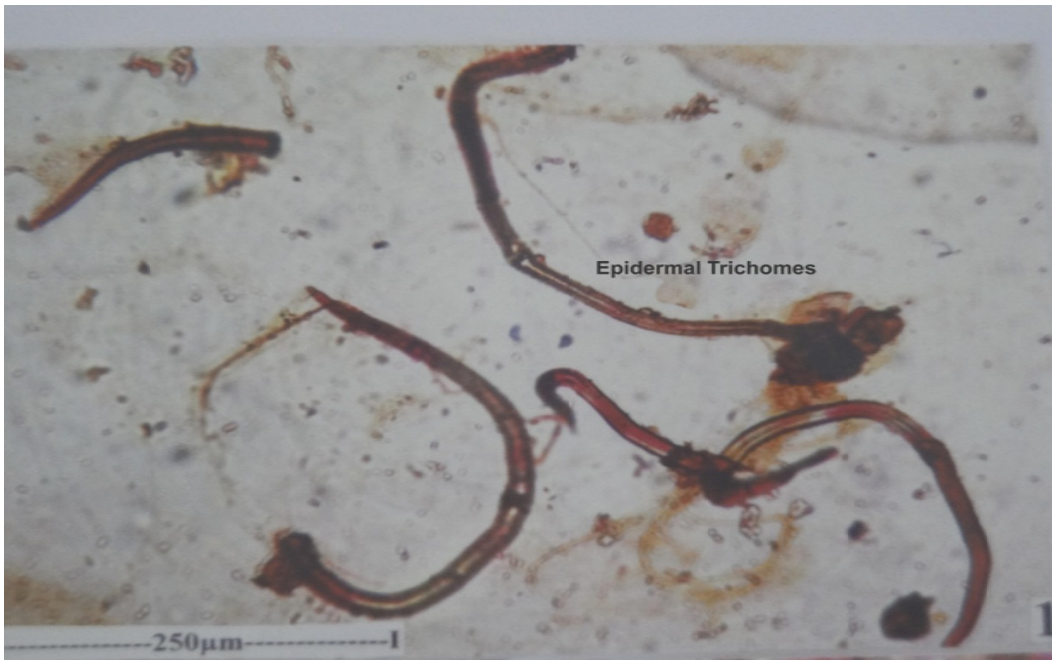


Fig.10.2: PARADERMAL SECTION OF THE EPIDERMIS

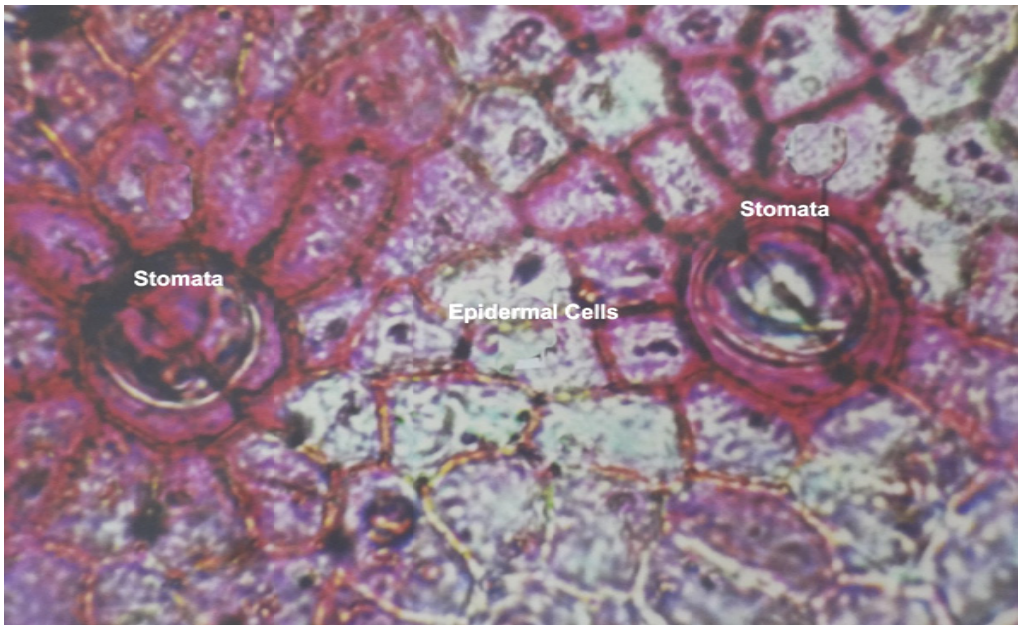
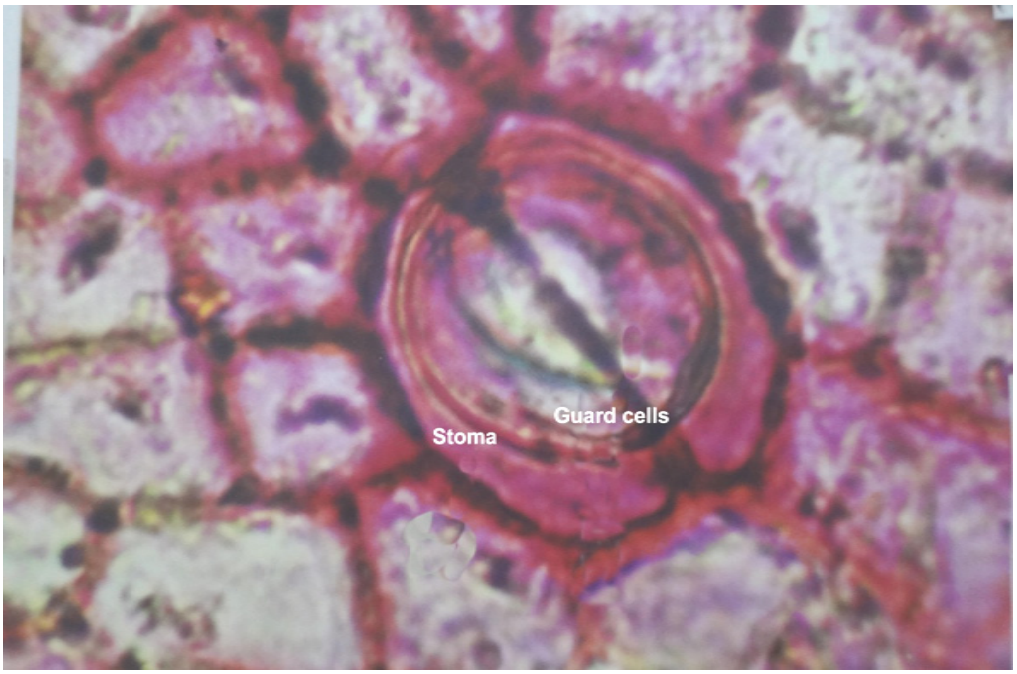


Fig.10.3:SINGLE STOMA ENLARGED



Lamina (**Fig.8.1**)

The lamina is 120µm thick. It consist of thick and wide, vertically oblong epidermal cells with prominent cuticle. The abaxial epidermis is thin and the cells are cylindrical. The epidermis bears dense non glandular and glandular trichomes. The mesophyll cells consist of adaxial palisade and abaxial spongy parenchyma cells. The palisade cells are single layered with thin vertical, long cylindrical cells and cylindrical, spherical or lobed parenchyma cells in spongy mesophyll tissue.

Venation pattern (Fig.9.1, 2)

The venation is densely reticulate. The primary, secondary and tertiary veins are thin, but distinct **(Fig.9.1).** The vein- islets are wide and well defined with prominent vein – boundaries. The vein- terminations, when present are long, slender and forked at the tip **(fig.9.2).**There are also long, unforked terminations.

Stomata (fig10.2, 3)

The stomata occur on the abaxial surface of the lamina. The stomata are circular measuring 50µm in diameter. Cell walls of the guard cells are thick walled. The stomata are diffuse in distribution. The epidermal cells small, angular and thick walled and straight. The stomata are anomocytic others are actinocytic having four or five radiating subsidiary cells. Stomata are clear and well defined.

Non glandular trichomes (fig.10.1)

Long, multicellular unbranched epidermal trichomes are abundant on the abaxial epidermis; the trichomes are two or three celled; narrow and pointed at the tip. The trichomes are mostly curved. The trichomes are 300-3500µm long.

SECTION-C

QUANTITATIVE ANALYTICAL MICROSCOPY [63, 64]

Quantitative analytical microscopy is useful for measuring the cell contents of the crude drugs, which help in their identification, characterization, and standardization. A clear idea about the identity and characteristic features of the drug can be obtained after several determinations; the characteristic's number obtained was noted and compared with a standard value to find out whether it is within the range.

Determination of stomatal number and stomatal index

Stomatal number: The average number of stomata/sq.mm area of each surface of a leaf epidermis is termed as stomatal number

Stomatal index: The stomatal index is the percentage which the number of stomata formed to the total number of epidermal cells, each stoma being counted as one cell.

To study the stomatal morphology (type of stomata), stomatal number and stomatal index of leaf, the leaf was subjected to epidermal peeling.

Procedure

The leaf was cleared by boiling with chloral hydrate solution or alternatively with chlorinated soda. The upper and lower epidermis was peeled out separately by means of forceps. The cleared leaf was placed on a slide and mounted in glycerin. A camera lucida and drawing board was placed and a stage micrometer was inserted for making the drawing scale. A square of 1mm was drawn by means of stage micrometer. The slide with cleared leaf (epidermis) was placed on the stage of the microscope and examined under 45X objective and 10X eye piece. The epidermal cell and stomata was traced. The numbers of stomata

present in the area of 1sq. mm. including the cell if at least half of its area lies within the square was counted. The result for each field was calculated and the average number of stomata per sq. mm was determined and their values were tabulated in **Table 1.**

For stomatal index, the glycerin mounted leaf peeling as mentioned above was made and circle (O) like mark for each stomata and a cross (X) like mark for each epidermal cells was marked on the chart paper. The stomatal index was calculated by using the formula,

Stomatal index = S/ (E + S) x 100

Where S was the number of stomata in 1sq mm area of leaf and E was the number of epidermal cells (including trichomes) in the same area of leaf. The values were tabulated in **Table 1.**

Determination of Vein Islets and Vein Terminations

The term vein islet is used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of conducting strands. The number of vein islet/sq.mm of leaf fragments is known as **vein islet number**. An ultimate free end or termination of a veinlet is termed as veinlet termination. The number of vein terminals present in one sq.mm area of leaf fragment is termed as **veinlet termination number**

Procedure

The fragment of leaf lamina with an area of not less than 1sq mm excluding the midrib and the margin of the leaf was taken. The fragments of leaf lamina were cleared by heating in a test tube containing chloral hydrate solution on a boiling water bath until clear. The cleared fragments were stained with saffranin solution and a temporary mount was prepared with glycerol solution. The stage micrometer placed on the microscopic stage, examined under 10X objective and 6X eye piece and an area of 1 sq mm square was drawn.

The cleared leaf piece was placed on the microscope stage, the vein islets and vein terminals included in the square was drawn. The number of vein islets and terminals within the square were counted. The results obtained for the number of vein islets and terminals in1sq mm were tabulated in **Table 1.**

Table 1: Quantitative analytical microscopical Parameters of the leaf of Gmelina *arborea*

S. No.	Parameters*	Values obtained*
1.	Stomatal number in lower epidermis	41.33±0.66
2.	Stomatal index in lower epidermis	27.33±0.33
3.	Vein islet number	35.67±0.88
4.	Vein termination number	19±0.58

*** mean of 3 readings ± SEM**

SECTION-D

PHYSICOCHEMICAL PARAMETERS

Determination of Moisture Content (Loss on Drying) [65, 66]

An accurately weighed 10g of powdered drug was placed in a tarred weighing bottle. Then the bottle was dried at 105^{o}C for 5h and weighed. The drying and weighing was continued at one hour intervals until the difference between the two successive weighing is not more than 0.25%. The loss on drying was calculated with reference to the amount of powder taken. The readings were tabulated in **Table 2.**

TABLE-2

PERCENTAGE OF LOSS ON DRYING

OBSERVATION NUMBER	LOSS ON DRYING (%)w/w
1	8.5
2	9.6
3	9.1
4	9.1
5	8.9
Minimum	8.5
Average	9.0
Maximum	9.6

Determination of Ash values [65, 66]

Ash Content [67]

The residue remaining after incineration is the ash content of crude drug, which simply represents inorganic salts naturally occurring in the drug or adhering to it or deliberately added to it as a form of adulteration. The total ash, acid insoluble ash and water soluble ash are generally determined.

Procedure

Determination of Total Ash

An accurately weighed 2g of air dried powdered drug was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^{o}C, upto 6hrs until free from carbon, then allowed to cool and weighed. The percentage of ash was calculated with reference to the air dried drug.

Determination of Acid Insoluble Ash

The total ash obtained from the previous procedure was mixed with 25ml of 2M hydrochloric acid and boiled for 5min in a water bath, and then the insoluble matter was collected in an ashless filter paper (Whatmann) and washed with hot water, dried and ignited for 15min at a temperature not exceeding 450^{o}C, cooled in a desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Determination of Water Soluble Ash

The total ash obtained from the previous procedure was mixed with 25ml of water and boiled for 5min in a water bath, and then the insoluble matter was collected in an ashless filter paper and washed with hot water, dried and ignited for 15min at a temperature not exceeding 450^{o}C, cooled in a desiccator and weighed. 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 drug. The values in respect of the total ash values, acid insoluble ash, water soluble ash and water insoluble ash were tabulated in **Table 3.**

Determination of sulphated Ash [68]

An accurately weighed 2g of air dried powdered drug in a tarred silica crucible which was previously ignited and cooled before weighing at a temperature not exceeding 450°C. The residue was moistened with 1ml of concentrated sulphuric acid, ignited at 800 ± 25°C until all black particles have disappeared. It was then cooled; again sulphuric acid was added and ignited. It was cooled and the percentage of sulphated ash was calculated with reference to air dried drug. The results obtained were presented in **Table.3.**

Table-3

ASH VALUE

OBSERVATION NUMBER	TOTAL ASH %	ACID INSOLUBLE ASH%	WATER SOLUBLE ASH%	SULPHATED ASH %
1	5	0.33		9.22
2	8	0.21		9.22
3	9.5	0.16		9.03
4	8		3.9	
5	8		3.5	
6	8.5		3.4	
Minimum	5	0.16	3.4	9.03
Average	7.8	0.23	3.6	9.16
Maximum	9.5	0.33	3.9	9.22

Determination of Extractive Values [66, 69]

The extractive values are the important factor to determine the amount of active principle or phytocostituents present in the plant materials, when extracted with suitable solvents. The extraction of crude plant materials with various solvents gives a solution containing different phytoconstituents. Composition of the phytoconstituents in a particular solvent depends upon the nature of drugs and the solvents used. This is an important tool for the analysis of crude plant materials for its identity, purity and quality.

Procedure

Determination of ethanol soluble extractive

An accurately weighed 5g of the air dried coarsely powdered drug was macerated with 100ml of ethanol in a closed flask for 24h, shaking frequently during the first 6h and allowed to stand for 18h. Thereafter filtered rapidly, taking precautions against loss of ethanol. Then evaporate 25ml of the filtrate to dryness in a tarred china dish at 105^{o}C and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air dried drug.

Determination of water soluble extractive:

An accurately weighed 5g of the air dried coarsely powdered drug was macerated with 100ml of chloroform water in a closed flask for 24h, shaking frequently during the first 6h and allowed to stand for 18h. Thereafter filtered rapidly, taking precautions against loss of chloroform water. Then evaporate 25ml of the filtrate to dryness in a tarred china dish at 105^{o}C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Determination of petroleum ether soluble extractive:

The procedure adopted under ethanol soluble extractive was followed using petroleum ether as a solvent.

Determination of chloroform soluble extractive:

The procedure adopted under ethanol soluble extractive was followed using chloroform as a solvent.

Determination of acetone soluble extractive:

The procedure adopted under ethanol soluble extractive was followed using acetone as a solvent.

Determination of ethyl acetate soluble extractive:

The procedure adopted under ethanol soluble extractive was followed using ethyl acetate as a solvent.

The extractive values obtained for different solvents were presented in **Table 4.**

TABLE-4

EXTRACTIVE VALUES

BY COLD MACERATION

S.NO	SOLVENTS USED	EXTRACTIVE VALUE (%W/W)
1	Petroleum ether	30.07
2	Ethyl acetate	6.55
3	Chloroform	4.87
4	Ethanol	11.31
5	Acetone	9.28
6	Water	26.38

Determination of Foaming Index [66]

Some plant materials when shaken with water cause persistent foam which may be attributed to the presence of saponins in that material. The foaming ability of an aqueous solution of plant materials and their extracts is measured in terms of foaming index.

Procedure

An accurate quantity of about 1g of the coarse plant material was weighed and transferred into an Erlenmeyer flask containing 100ml of boiling water. The flask was boiled at moderate heat for 30min. The solution was cooled and filtered into a 100ml volumetric flask and sufficient distilled water was added to dilute to volume. The solution was poured into ten stoppered test tubes in successive portions of 1ml, 2ml etc. upto 10ml and the volume of the liquid in each tube was adjusted with water upto 10ml. The tubes were then stoppered and shaken in a length wise motion for 15sec (two shakes/sec) and allowed to stand for 15min. The height of foam was measured. If the height of the foam in every tube was less than 1cm, the foaming index was less than 100. If the height of foam was measured 1cm in

any test tube, the volume of the plant material decoction in that tube (A) was used to determine the index. If the height of the foam was more than 1cm in every tube, the foaming index was over 1000. In this case, the determination was repeated using a new series of dilution of the decoction in order to obtain a result. The foaming index was calculated by using the following formula 1000/A where A was the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm was observed.

The height of the foam in every tube was less than 1cm hence the foaming index was found to be less than 100.

SECTION-E

POWDER ANALYSIS

The powdered crude drug analysis was aimed to study and also to assess the quality of herbal drugs for therapeutic value which are generally studied by classical Pharmacognostical studies. The authenticity of herbal drugs was confirmed by comparison of their powder characteristics.

Procedure

a. Fluorescence analysis

The fluorescence analysis of the various extracts of *Gmelina arborea* was carried out by using the method of Chase and Pratt (1949) [69]. The observations were tabulated in **Table 5.**

Table 5: Fluorescence analysis of various extract of *Gmelina arborea* leaves

Extract	Day light	UV light(254nm)	UV light (366nm)
Pet. Ether	Light brown	Light green	Green
Ethyl acetate	Green	Dark green	Orange
Aqueous	Light brown	light green	Green
Chloroform	Brown	Brownish green	Orange
Acetone	Green	Dark green	Orange
Ethanol	Green	Dark green	Orange

b. Powder microscopy

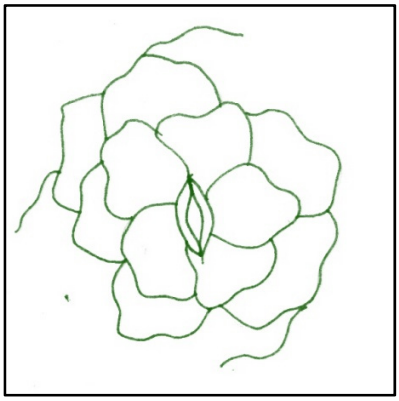
The dried plant material was powdered and the powder was passed through sieve no.6 for the study of powder microscopy. Chloral hydrate, water, iodine, phloroglucinol and hydrochloric acid (1:1), lacto phenol etc.were employed as mounting medium. The pictorial representations were presented in **Fig.11.**

The powder microscopy of leaves shows the following characters

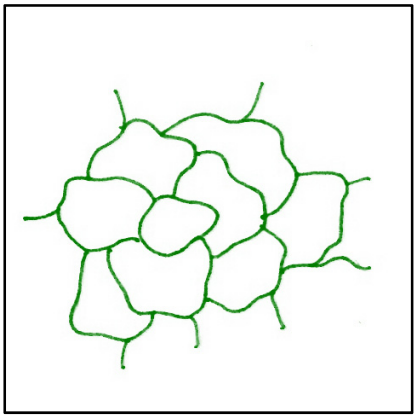
- Multicellular-uniseriate unbranched epidermal trichomes
- Fragments of spongy parenchyma
- Fragments of palisade cells
- Epidermal cells with actinocytic stomata
- Spiral xylem vessel

Fig. 11 : POWDER MICROSCOPY OF *Gmelina arborea Roxb.*

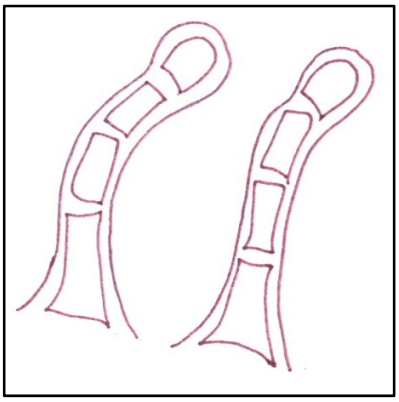
Epidermal cells with
Actinocytic stomata



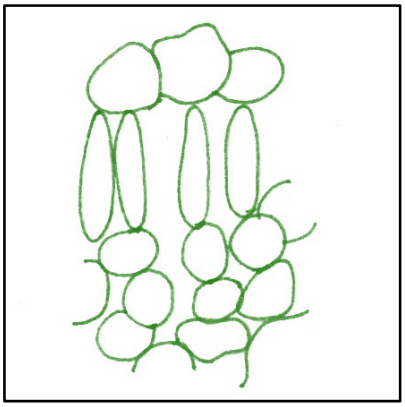
Parenchyma cells



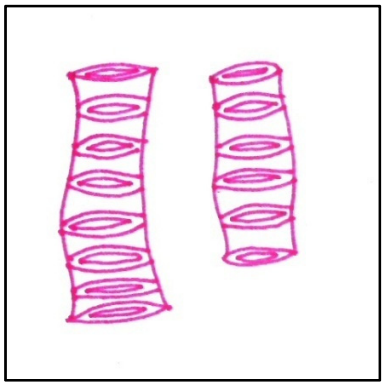
Nonglandular Trichomes



Palisade cells



Xylem Vessels



PHYTOCHEMICAL EVALUATION

CHAPTER-VI

PHYTOCHEMICAL EVALUATION

Phytochemical (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. [70] They protect plants from disease and damage and contribute to the plant's colour, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals [71, 72]. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant.

SECTION-A

ORGANOLEPTIC EVALUATION

Nature – Coarse powder

Colour – Light Green

Odour – Characteristic odour

Taste – Slightly bitter taste

SECTION-B

PRELIMINARY PHYTOCHEMICAL SCREENING

The shade dried and coarsely powdered leaves of *Gmelina arborea* and its various extracts were subjected to the following chemical tests. [73-75]

1. Test for sterols

The powdered leaf was first extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols.

a.Salkowski's Test

A few drops of concentrated sulphuric acid were added to the chloroform solution, shaken well and set aside. The lower chloroform layer of the solution turning red in color indicates the presence of sterols.

b.Liebermann – Burchard's Test

To the chloroform solution, a few drops of acetic anhydride and 1ml of concentrated sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring appears and the upper layer turned green colour indicating the presence of sterols.

2. Test for carbohydrates

a. Molisch's Test

The aqueous extract of the powdered leaf was treated with few drops of alcoholic-α-naphthol solution and sulphuric acid. A purple colour indicates the presence of carbohydrates.

b. Benedict Test:

The aqueous extract of the powdered leaf was treated with Benedict's reagent and heated on a boiling water bath for half an hour. Red precipitate indicates the presence of free reducing sugars.

c. Fehling's Test

The aqueous extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. A red precipitate indicates the presence of free reducing sugars

3. Test for Proteins

a. Millon's Test

A small quantity of aciduous–alcoholic extract of the powdered drug was heated with Millon's reagent. White precipitate turning red on heating indicates the presence of proteins.

b. Biuret Test

To one portion of aciduous–alcoholic extract of the powdered drug, 1ml of 10% sodium hydroxide solution was added, followed by one drop of dilute copper sulphate solution. Violet color indicates the presence of proteins.

4. Test for fixed oil

Small quantity of leaf powder was pressed between two filter papers. No oil stain indicates the absence of fixed oils.

5. Test for Alkaloids

About 2g of the powdered material was mixed with 1g of calcium hydroxide and 5ml of water into a smooth paste and set aside for 5min. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200ml of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To the residue, 5ml of dilute hydrochloric acid was added. The solution was divided into four parts and to the 2ml of each part, the following reagents were added and the colour noted below indicates the presence of alkaloids.

a) Mayer's Reagent - Cream precipitate produced

b) Dragendorff's Reagent - Reddish brown precipitate produced

c) Hager's Reagent - Yellow precipitate

d) Wagner's Reagent - Reddish brown precipitate

Test for purine group (Murexide test)

The residue obtained after the evaporation of chloroform as described above was treated with 1ml of hydrochloric acid in a porcelain dish and 0.1gm of potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapour of dilute ammonia solution. No purple colour indicates the absence of purine group of alkaloids.

6. Test for Glycosides

a. Borntrager's Test

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate, benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. No pink color in the ammonical layer shows the absence of anthraquinone glycosides.

b.Modified Borntrager's Test

About0.1g of the powdered drug was boiled for 2min 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. No pink color in ammonical layer shows the absence of anthraquinone glycosides.

Test for Cardiac Glycosides (for deoxysugar)

a. Keller Kiliani Test

About 1g of the powdered leaf was boiled with 10ml of 70% alcohol for 2min, cooled and filtered. To the filtrate 10ml of water and 5 drops of solution of leadsubacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3ml of glacial acetic acid. To this, 2 drops of ferric chloride solution was added. Then 3ml of concentrated sulphuric

acid was added along the sides of the test tube carefully and observed. No reddish brown layer indicates the absence of deoxysugar of cardiac glycoside.

b. Legal test

The powdered leaf was dissolved in pyridine, sodium nitroprusside solution was added to it and made alkaline. No pink or red colour indicates the absence of cardiac glycosides.

c. Baljet test

To the powdered leaf sodium picrate solution was added. No yellow or orange colour indicates the absence of cardiac glycosides.

Test for coumarin glycosides [76]

A small amount of powdered drug was placed in test tube and covered with filter paper moistured with 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. No green colour fluorescence was observed indicating the absence of coumarin glycoside.

7. Test for Saponins

About 0.5g of the powdered drug was boiled gently for 2min with 20ml of water and filtered while hot and allowed to cool. 5ml of the filtrate was then diluted with water and shaken vigorously. Frothing indicates the presence of saponins.

8. Test for Tannins and phenolic compounds

a) A small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added. A bluish black colour was produced indicating the presence of tannins.

b) To a small quantity of aqueous solution of the powder, a few drops of bromine water were added. Decolourisation of bromine water indicates presence of tannins.

9. Test for Flavonoids

a.Shinoda test:

A little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red colour indicates the presence of flavonoids.

b. Alkali Test

To the small quantity of test solution, 10% aqueous sodium hydroxide solution was added. Yellow orange color indicates the presence of flavonoids.

c. Acid Test

To the small quantity of test solution, few drops of concentrated sulphuric acid was added. Yellow orange color indicates the presence of flavonoids.

10. Test for terpenoids

The powdered leaf was shaken with petroleum ether and filtered. The filtrate was evaporated and the residue obtained was dissolved in small amount of chloroform and to the chloroform solution tin and thionyl chloride were added. No pink color indicates the absence of terpenoids.

11. Test for volatile oil

About 100gm of fresh leaves were taken in a volatile oil estimation apparatus and subjected to hydro distillation for 4 hrs. No volatile oil was observed indicating the absence of volatile oil.

12. Test for Mucilage

A few ml of aqueous extract was prepared from the powdered drug and treated with ruthenium red. A pinkish red colour indicates the presence of mucilage. The preliminary phytochemical evaluation of the leaf powder and the various extracts of the leaves of *Gmelina arborea* were carried out and the results obtained were presented in **Table 6 and Table7.**

Table 6: Preliminary phytochemical screening for the leaf powder of *Gmelina arborea*

S.NO	TEST	RESULTS
1.	**TEST FOR STEROLS**	
	a. Salkowski's test	+
	b. Libermann- burchard's test	+
2.	**TEST FOR CARBOHYDRATES**	
	a.Molisch's test	+
	b. Benedict's test	+
	c. Fehling's test	+
3.	**TEST FOR PROTEINS**	
	a.Millon'stet	+
	b. Biuret test	+
4.	**TEST FORFIXEDOILS**	-
5.	**TEST FOR ALKALOIDS**	
	a. Mayer's reagent	+
	b. Dragendorff's reagent	+
	c. Hager's reagent	+
	d. Wagner's reagent	+
6.	**TEST FOR GLYCOSIDES**	
	a. Anthraquinone glycosides	
	i) Borntrager's test	-
	ii) Modified Borntrager's test	-
	b. Cardiac glycosides	
	i) Keller Kiliani test	-
	ii) Baljet test	-
7.	**TEST FOR SAPONINS**	+
8.	**TEST FOR TANNINS**	
	a) $FeCl_3$ test	+
	b) Lead acetate test	+
9.	**TEST FOR FLAVONOIDS**	
	a. Shinoda test	+
	b. Alkali test	+
10.	**TEST FOR TERPENOIDS**	-
11.	**TEST FOR VOLATILE OILS**	-
12.	**TEST FOR MUCILAGE**	+

(+) indicates positive reaction (-) indicates negative reaction

Table 7: Preliminary Phytochemical Screening for the various extracts of leaf powder of *Gmelina arborea Roxb.*

Tests	Petroleum ether extract	Ethyl acetate extract	Chloro form extract	Methanol Extract	Ethanol Extract	Aqueous Extract
1.Test for sterols						
a.Salkowski's test	+	+	+	-	-	-
b.Liebermann-Burchard's test	+	+	+	-	-	-
2.Test for carbohydrates						
a. Molisch's test	-	-	-	+	+	+
b. Fehling's test	-	-	-	+	+	+
c. Benedict's test	-	-	-	+	+	+
3.Test for Proteins						
a. Millon's test	-	-	-	-	-	+
b. Biuret test	-	-	-	-	-	+
4.Test for Fixed oils	-	-	-	-	-	-
5.Test for Alkaloids						
a. Mayer's reagent	-	-	-	+	+	-
b. Dragendorff's reagent	-	-	-	+	+	-
c. Hager's reagent	-	-	-	+	+	-
d. Wagner's reagent	-	-	-	+	+	-
6.Test for Glycosides						
a. Anthraquinone glycosides						
i) Borntrager's test	-	-	-	-	-	-
ii) Modified Borntrager's test	-	-	-	-	-	-
b. Cardiac glycosides						
i) Keller Kiliani test	-	-	-	-	-	-
ii) Baljet test	-	-	-	-	-	
iii) Coumarin glycosides	-	-	-	-	-	-
7. Test for Saponins	-	-	-	+	+	+
8.Test for Tannins						
a. $FeCl_3$ test	-	+	-	+	+	+
b. Lead acetate test	-	+	-	+	+	+
9.Test for Flavonoids						
a. Shinoda test	-	-	-	+	+	-
b. Alkali test	-	-	-	+	+	-
c.Acid test						
10. Test for Terpenoids	-	-	-	-	-	-
11. Test for Volatile Oils	-	-	-	-	-	-
12. Test for Mucilage	-	-	-	-	-	+

(+) indicates positive reaction **(-) indicates negative reaction**

From the table, it can be observed that the ethanol extract showed the presence of carbohydrates, saponins, alkaloids, flavonoids, Tannin and phenolic compounds while the petroleum ether and chloroform extracts showed the presence of sterols. The ethyl acetate extract showed the presence of sterols and tannins. The aqueous extract showed the presence of carbohydrate, protein, saponin, tannin and mucilage.

SECTION-C

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS [77-79]

The quantitative estimation of phytoconstituents is very essential for identifying and quantifying the phytochemicals present in the medicinal plants which is important for therapeutic action. A particular group of compound present in the crude extracts can be quantified by means of using standard or reference marker compound and then reporting them as equivalent to that much amount of compound present in that extract as per standard compound. All the phenolic compounds are oxidised by the Folin-Ciocalteu Reagent.

1. Determination of total phenolic content

Principle

The total phenol content of *Gmelina arborea* was determined by the Folin-Ciocalteu colorimetric method (Singleton *et al*., 1999). The Folin Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate. The method measures the amount of substance needed to inhibit the oxidation of the reagent.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

10% sodium carbonate

1N Folin-Ciocalteu reagent (diluted with equal volume of water and made 1N)

Procedure

0.5ml and 1ml of ethanolic extracts of *Gmelina arborea* (1mg/ml) were taken in separate test tubes. 0.5ml of FolinCiocalteu reagent (1N) was added and allowed to stand for 15 mins. Then 1ml of 10% sodium carbonate solution was added. Finally the mixtures were mixed with distilled water and made upto10ml, allowed to stand for 30 minutes at room temperature and total phenols were determined by spectrophotometrically at 760nm. The reaction mixture without sample was used as blank. Gallic acid at different concentration i, e.20, 40, 60, 80, 100µg/ml was taken and treated in the same manner and the absorbance was measured at 760nm.

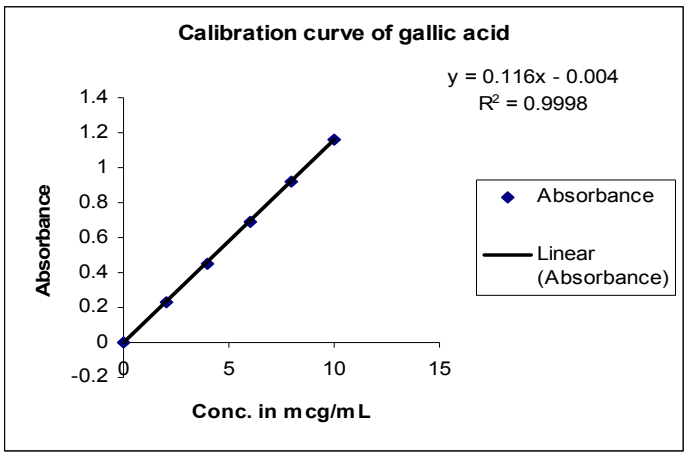
A calibration curve was generated by plotting concentration versus absorbance (**Fig.12**). A regression equation was constructed and using the equation, the total phenol content of ethanolic extracts of *Gmelina arborea* was expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g) in **Table8.**

Table8: Total phenolic content in ethanolic extract of *Gmelina arborea* in terms of gallic acid equivalents

S. No.	Conc. of gallic acid in µg/ml	Absorbance at 760nm	Conc. of ethanolic extract in µg/ml	Absorbance at 760nm*	Amount of total phenolic content in terms mgGAE/g of extract*
1	20	0.229 ± 0.010	50	0.258±0.02	49.46±4.01
2	40	0.452 ± 0.006	100	0.508±0.03	99.4±5.64
3	60	0.695 ± 0.005		**Average**	**74.43±4.825**
4	80	0.918 ± 0.031			
5	100	1.162 ± 0.028			

*** mean of three readings ±SEM**

Fig.12: Calibration curve of gallic acid



The linear regression equation was found to be y = 0.116x-0.004 while the correlation was found to be 0.9998. The amount of phenolic content present in the extract in terms mg GAE/g of extract was found to be 74.43±4.825by using the above linear regression equation.

2. Determination of total flavonoid content [80-82]

Principle

The aluminum chloride colorimetric technique was used for estimation of total flavonoid estimation. Aluminum ions form stable complexes with C4 keto group and either to C3 or C5 hydroxyl groups of flavones and flavonols in acidic medium. It also forms acid labile complexes with ortho hydroxyl groups in the A or B rings of flavonoids. These complexes showed a strong absorption at 415nm which is used for the estimation of flavonoids.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

10% aluminum chloride

1M potassium acetate

Procedure

An aliquot quantity of quercetin was dissolved in ethanol to get a stock solution of 1mg/ml. Further dilutions were made to get concentrations ranging from 20-100µg/ml. 1ml of the above standard solutions were taken in different volumetric flasks, 0.1ml of aluminum chloride solution, 0.1ml of potassium acetate solution and 2.8ml of ethanol were added and the final volume was then made up to 5ml with distilled water. After 20min the absorbance was measured at 415nm. A sample without aluminium chloride was used as a blank. From the absorbance obtained, a calibration curve was constructed by plotting concentration versus absorbance of quercetin (**Fig.13)**.0.5ml and 1ml of ethanolic extract of *Gmelina arborea* leaves of(1mg/ml) were taken and the reaction was carried out as above and the absorbance was measured at 415nm after 20min and the readings were tabulated in **Table9.** The amount

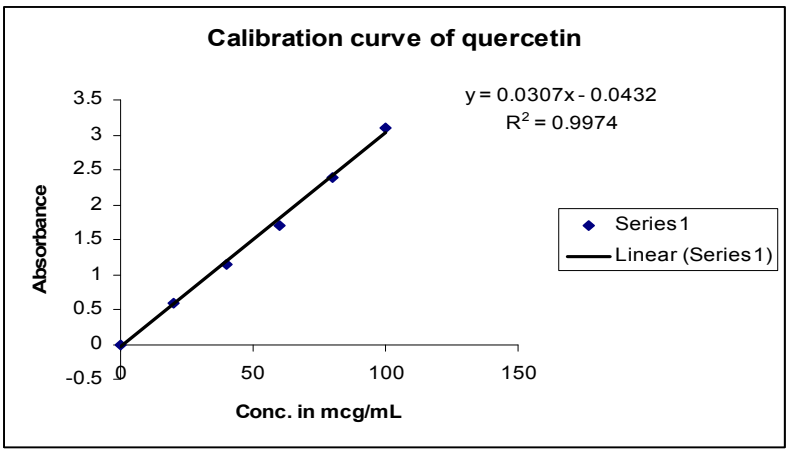
of flavonoids present can be determined by linear regression analysis. The total flavonoid content was expressed as mg quercetin equivalents /g of extract.

Table9: Total flavonoid content per gram of extract in terms of quercetin by aluminium chloride method

S. No.	Conc. of quercetin in µg/ml	Absorbance at 700nm	Conc. of methanolic extract in µg/ml	Absorbance at 700nm	Amt of total flavonoid content in terms mg quercetin equivalent/ g of extract
1	20	0.589±0.01	100	0.107 ± 0.01	48.93±2.13
2	40	1.151±0.04	200	0.286 ± 0.05	53.72±7.52
3	60	1.710±0.09		**Average**	**51.32±4.82**
4	80	2.390±0.03			
5	100	3.112±0.03			

***mean of three readings ± SEM**

Fig.13: Calibration curve of quercetin



The linear regression equation was found to be y = 0.0307x-0.0432 while the correlation was found to be 0.9974. The amount of flavonoid content present in the ethanolic extract of *Gmelina arborea* in terms mg quercetin equivalent/g of extract was found to be 51.32±4.82mg/g of extract by using the above linear regression equation.

3. Determination of total tannin content

Principle

Total tannin content of ethanolic extracts *Gmelina arborea* was determined by Folin Denis reagent method (Schanderl, 1970). [83]

Tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a highly blue coloured solution [84]. The intensity is measured in a spectrometerically at 700nm

Reagents

a) Folin Denis Reagent (sodium tungstate 100g and phosphomolybdic acid 20gm were dissolved in distilled water 750ml along with phosphoric acid 50ml. The mixture was refluxed for 2 hr and volume was made up to 1 litre with distilled water)
b) Sodium carbonates solution (10%)

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Procedure

0.2ml and 0.4ml of (1mg/ml) ethanolic extracts of *Gmelina arborea* was taken in separate test tubes and mixed with distilled water to made up to the volume of 1ml. Then add 0.5ml of Folin Denis reagent and allowed to stand for 15 mins, then 1ml of 10% sodium carbonate solution was added and the mixture was mixed with distilled water and made up to

10ml, allowed to stand for 30mins at room temperature and the tannin content was determined spectrophotometrically at 700nm.

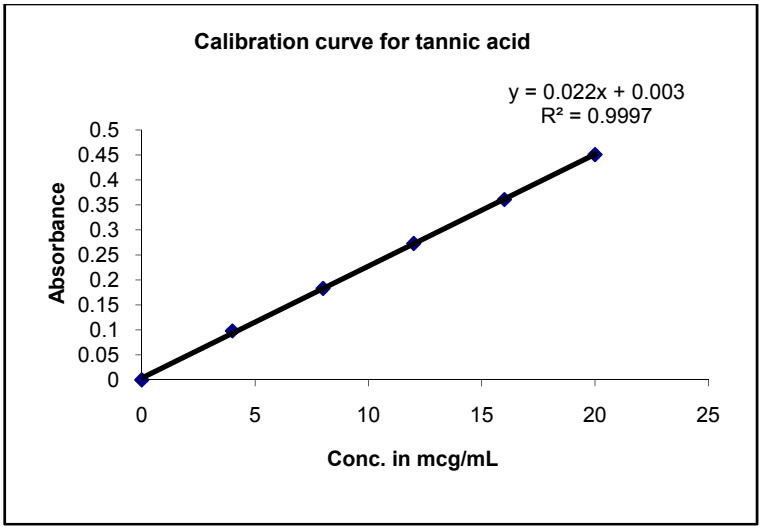
The calibration curve was generated by preparing tannic acid at different concentration (20, 40, 60, 80,100µg/ml). The reaction mixture without sample was used as blank. The total tannin content in the ethanolic extract of *Gmelina arborea* was expressed as milligrams of tannic acid equivalent per gm of extract in **Table.10** and **Fig.14.**

Table 10: Total tannin content in ethanolic extract of *Gmelina arborea* in terms of tannic acid equivalents

S. No.	Conc. of tannic acid in µg/ml	Absorbance at 415nm	Conc. of ethanolicextract in µg/ml	Absorbance at 415nm	Amt of total tannic acid content in terms mg quercetin equivalent/ g of extract
1	20	0.589±0.01	20	0.115 ± 0.004	22.8±0.92
2	40	1.151±0.04	40	0.219 ± 0.018	43.66±3.58
3	60	1.710±0.09		**Average**	**33.23±2.25**
4	80	2.390±0.03			
5	100	3.112±0.03			

***mean of three readings ± SEM**

Fig 14: **calibration curve for tannic acid**



The linear regression equation was found to be y = 0.022x-0.003 while the correlation was found to be 0.9997. The amount of tannin content present in the ethanolic extract of *Gmelina arborea* in terms mg tannic acid equivalent/g of extract was found to be 33.23±2.25mg/g of extract by using the above linear regression equation.

SECTION-D

CHROMATOGRAPHY [85]

Chromatography may be defined as the method of separating a multi component mixture into individual components through equilibrium distribution between two phases. The technique of chromatography was first invented by M.Tswett, a botanist in 1906 in Warsaw. Tswett termed chroma and graphos means "colour" and "writing" respectively. The technique is mainly based on the differences in the rate at which components of mixture move through a porous medium (called stationary phase) under the influence of some solvent or gas (called mobile phase)**.**

The steps involved in the chromatography

- Adsorption or retention of substances or substances on the stationary phase
- Separation of adsorbed substances by mobile phase
- Recovery of separated substances by continuous flow of mobile phase the method being called elution
- Quantitative and qualitative analysis

Types of chromatography

The chromatographic methods can be classified based on the nature of the stationary and mobile phases used. If the stationary phase is a solid, the principle of separation is called as adsorption chromatography and if the stationary phase is a liquid, it is termed as partition

chromatography. The various types of chromatography include paper chromatography (PC), thin layer chromatography (TLC), column chromatography (CC), gas chromatography (GC), high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) are widely used for plant analysis.

Thin Layer Chromatography [86]

Thin layer chromatographic technique is most widely used for the separation and identification of active components from plant sources. The principle of separation involved here is adsorption. The adsorbent should show a maximum selectivity toward the substances being separated so that the differences in rate of elution will be large. For the separation of any given mixture, some adsorbents may be too strongly adsorbing or too weakly adsorbing. Depending on the distribution coefficients, the compounds are distributed on the surface of the adsorbent. The compound, which is readily soluble but not strongly adsorbed, moves up along with the solvent and that not so soluble but more strongly adsorbed move up less readily leading to the separation of compounds.

Preparation of TLC Plates

The adsorbent (silica gel G) slurry was prepared in water in the ratio of (1: 2). The glass plates (20cm x 5cm) were cleaned and laid in a row as a template, the suspension was poured into Stahl TLC spreader, which was adjusted to 0.25mm thickness and coated in a single passage of the spreader over them. These plates were air dried and activated in hot air oven at 105ºC for 30min and kept in a dessicator. The plates were used as the stationary phase or Pre-coated aluminum plates coated with silica gel G F_{254} (Merck) were also used for analysis.

Sample application

The sample was prepared by dissolving the ethanolic extract of *Gmelina arborea* in ethanol to get 5mg/ml. The sample was applied as a spot with the help of capillary tube.

Development of the chromatogram

The TLC plates were developed in a chromatographic tank by using different mobile phase's viz., solvent system I, solvent system II. The plates were allowed to develop ¾ of the length and then removed. The solvent front was immediately marked and the plates were allowed to dry and then it was examined by visually, under UV light at 254 and 365nm.The results were presented in **Table11.**

Stationary phase **:** Silicagel G

Mobile phase **:** 1.Ethyl acetate: Benzene (2:8)

2. Chloroform: Ethyl acetate (60:40)

Detecting agent **:** Visual and UV light

The R_f value of the spots obtained were calculated using the formula

$$R_f \text{value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

The photographic representation of the chromatogram presented in **Fig.15** and the R_f values of the spots at 254and365nm were presented in **Table11.**

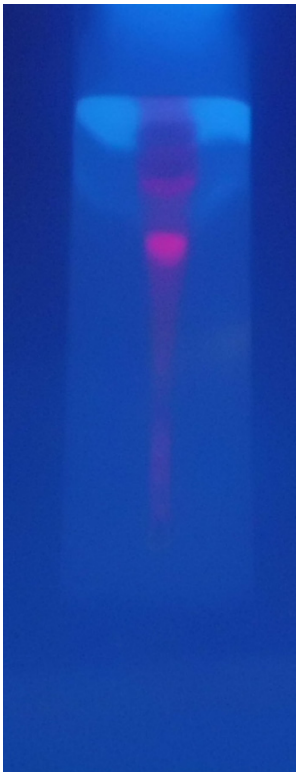
Table 11: TLC of the ethanolic extract of *Gmelina arborea*

S. No	Solvent system	Detecting agent	No of spots	Colour of spots	R_f values
1.	Ethyl acetate: Benzene (2:8)	Under UV light at 365nm	I	Yellow	0.37
			II	Orange fluorescence	0.8
			III	Yellow	0.92
2.	Chloroform: Ethyl acetate(60:40)	Under UV light at 365nm	I	Yellow	0.23
			II	Orange fluorescence	0.8
			III	Yellow	0.92

TLC OF ETHANOLIC EXTRACT OF *Gmelina arborea* leaves

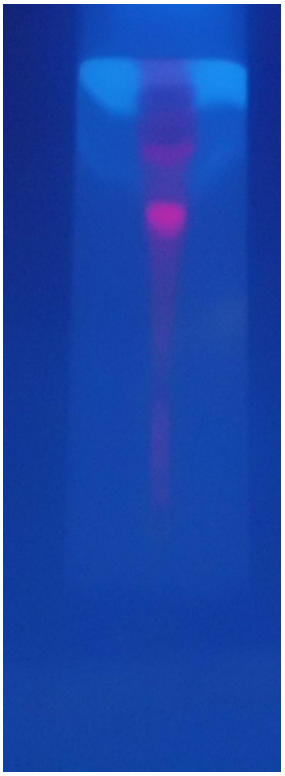
Solvent system-I

@365 nm



Solvent system-II

@365 nm



The extract showed fluorescent spots at an R_f value of 0.37, 0.8, 0.92 and 0.23, 0.8, 0.92 when viewed under UV at 365nm after development in the mobile phases.

High Performance Thin Layer chromatography [87]

High-performance thin-layer chromatography (HPTLC) has been emerged as an important tool for the qualitative, semi quantitative, and quantitative phytochemical analysis of the herbal drugs and formulations. This includes developing TLC fingerprinting profiles and estimation of biomarkers. This review has an attempt to focus on the theoretical considerations of HPTLC and some examples of herbal drugs and formulations analyzed by HPTLC. Standardized manufacturing procedures and suitable analytical tools are required to establish the necessary framework for quality control in herbals. Among those tools, high-performance liquid chromatography (HPLC), high-performance thin layer chromatography (HPTLC), and capillary electrophoresis are the most widely used to establish reference fingerprints of herbs, against which raw materials can be evaluated and finished products can be assayed. High-performance thin-layer chromatography, also known as planar chromatography, is a modern technique with high separation power and reproducibility superior to classical TLC. Main difference of HPTLC and TLC is particle and pore size of sorbents.

Special features of HPTLC

1. Simultaneous processing of sample and standard – better analytical precision and accuracy and less need for internal standard
2. Several analysts can work simultaneously
3. Lower analysis time and less cost per analysis
4. Low maintenance cost
5. Simple sample preparation – can handle samples of divergent nature
6. No prior treatment for solvents like filtration and degassing

7. Low mobile-phase consumption per sample

8. No interference from previous analysis – fresh stationary and mobile phases for each analysis, no contamination

9. Visual detection possible – open system

L0.Non-UV-absorbing compounds detected by post chromatographic derivatization

HPTLC evaluation of ethanolic extract of *Gmelina arborea*

Instrument used : CAMAG make HPTLC.

Software : win CATS 1.4.3

Sample Applicator : Linomat 5.

Detection : @520nm, @254nm & @366nm in Densitometry TLC Scanner 3

Sample preparation : sample was dissolved in 1 ml methanol

Stationary Phase : HPTLC plates silica gel 60 F 254.

Mobile Phase : Ethyl acetate: Toluene (2:8)

Sample Solution : 2µl sample is applied as 8mm band.

The visualization of the TLC plate of ethanolic extract of *Gmelina arborea* at 254 and 365nm and white light was presented in **Fig.16.** The 3D display of the chromatogram and the peak area of the corresponding area under the curve were presented in **Table12.**

High Performance Thin layer Chromatography

The visualization of the TLC plate of ethanolic extract of *Gmelina arborea* at 254nm and 366nm is presented in **Fig.16.** The photo of plate at 254nm shows the presence of 8 spots while at 366nm shows the presence 9 spots. The 3D display of the fingerprint profile and the peak display of ethanolic extract of *Gmelina arborea* at 254nm and 366nm is presented in **Figs.17&18.** The display at 254nm shows the presence of 8 peaks while at 366nm shows the

Fig. 16: Visualization at 254nm and 366nm and white light

White light **@ 254nm** **@366nm**

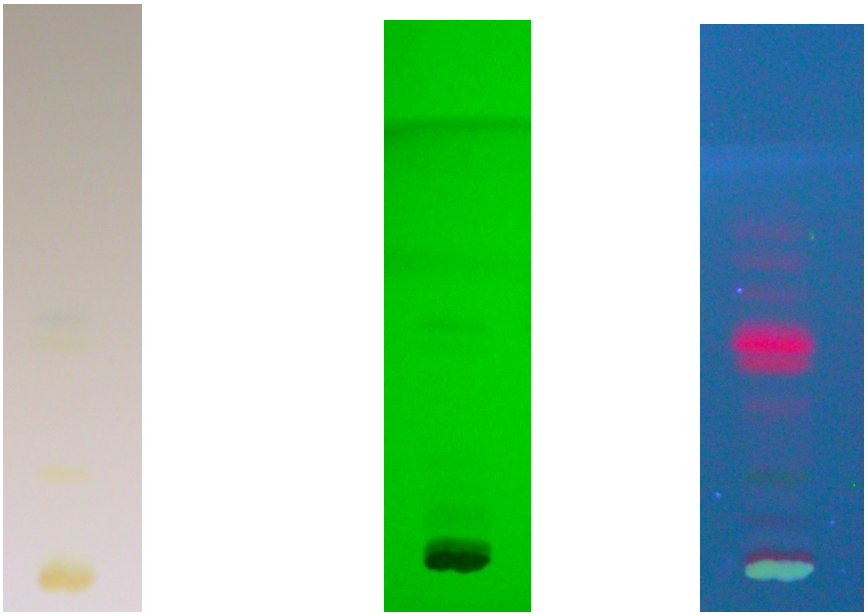
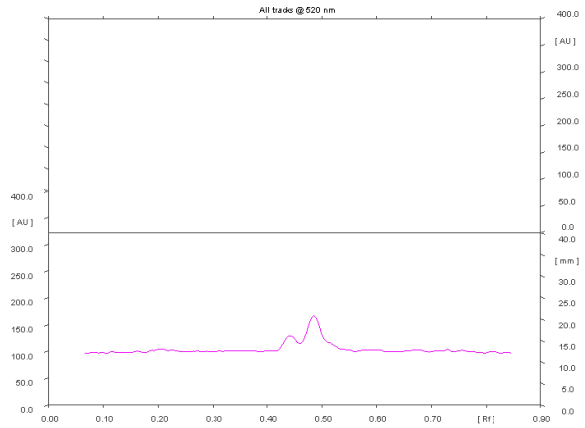
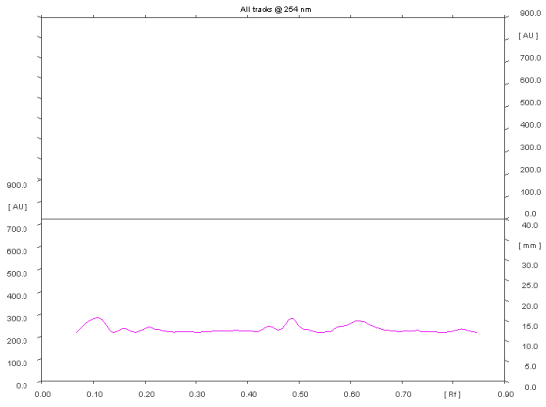


Fig. 17: 3D Display of the fingerprint profile at 254nm and 366nm

White light



@ 254nm



@ 366nm

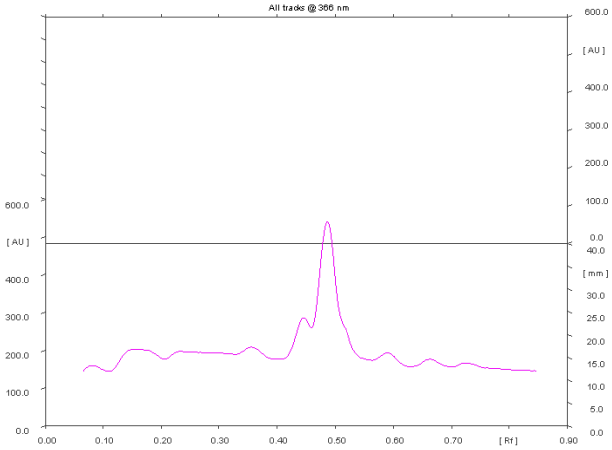
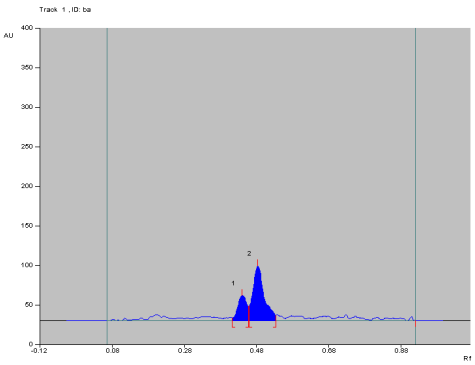
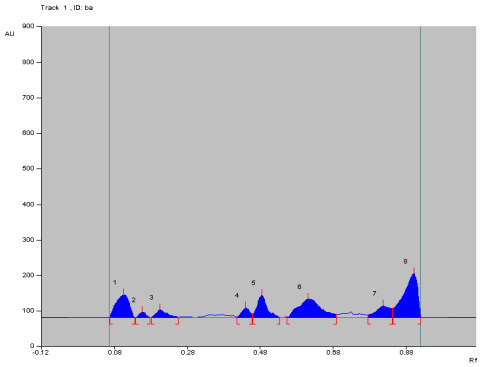


Fig. 18: Peak display of ethanolic extract of *Gmelina arborea* at 254nm and 366nm.

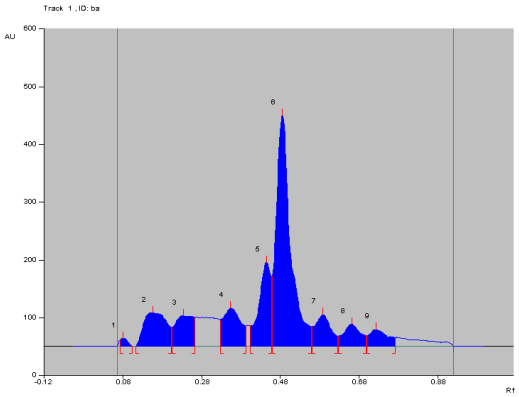
White light



254nm



@ 366nm



presence 9 peaks. The R_f values of the peaks along with the area under the curve for each peak at 254 and 366 nm were tabulated in **Table12.**

Table 12: R_f values and area under the curve for each peak at 254 and 366nm

S.No	White light		@ 254nm		@ 366nm	
	Rf value	**Area (AU)**	**Rf value**	**Area (AU)**	**Rf value**	**Area (AU)**
1	0.44	630.2	0.11	1782.2	0.08	190.8
2	0.49	1684.8	0.16	223.0	0.16	2626.7
3			0.21	506.7	0.23	1916.3
4			0.44	468.4	0.36	2342.5
5			0.49	1305.4	0.45	3427.8
6			0.61	2528.4	0.49	11221.6
7			0.82	1003.0	0.59	1671.9
8			0.90	3656.7	0.66	1304.7
9					0.73	1063.1

The HPTLC finger print profile of the ethanolic crude plant extract of *Gmelina arborea* showed various peaks with the Rf values indicates that the plant contains many medicinally active compounds were responsible for its therapeutic activity.

PHARMACOLOGICAL EVALUATION

CHAPTER-VII

PHARMACOLOGICAL EVALUATION

The ethanolic extract of *Gmelina arborea* was subjected to pharmacological screening and the following activities were carried out namely in-vitro antioxidant activity, anti-arthritic activity and in-vivo anti-epileptic activity.

SECTION-A

IN-VITRO ANTI OXIDANT ACTIVITY

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, Phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the monophenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are

the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers**.** [88]

Method 1: Free radical Scavenging activity using diphenylpicrylhydrazyl (DPPH) free radical

The free radical scavenging activity of the extracts is evaluated by assessing their ability to reduce the colour of DPPH in ethanol according to Brand Williams [89]. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts [90].

Principle

A simple method that has been developed to determine the antioxidant activity of plants utilizes the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured [91].

$$DPPH^{\cdot} + AH \rightarrow DPPH - H + A^{\cdot}$$

Instrument

Shimadzu UV Visible spectrometer, Model 1800

Reagents

0.1mM Diphenylpicrylhydrazyl Radical in ethanol.

Procedure [91, 92]

A stock solution of 1mg/ml concentration of ethanolic extract of *Gmelina arborea was* prepared. To 1ml of various concentrations (40 to 200µg/ml) of test samples, 4ml of DPPH was added. Control was prepared without sample in an identical manner. DPPH was

replaced by ethanol in case of blank. The reaction was allowed to complete in the dark for about 30min. Then the absorbance was measured at 517nm. Vitamin C was used as standard. The percentage scavenging was calculated using the formula

[(Control-Test)/Control] x 100

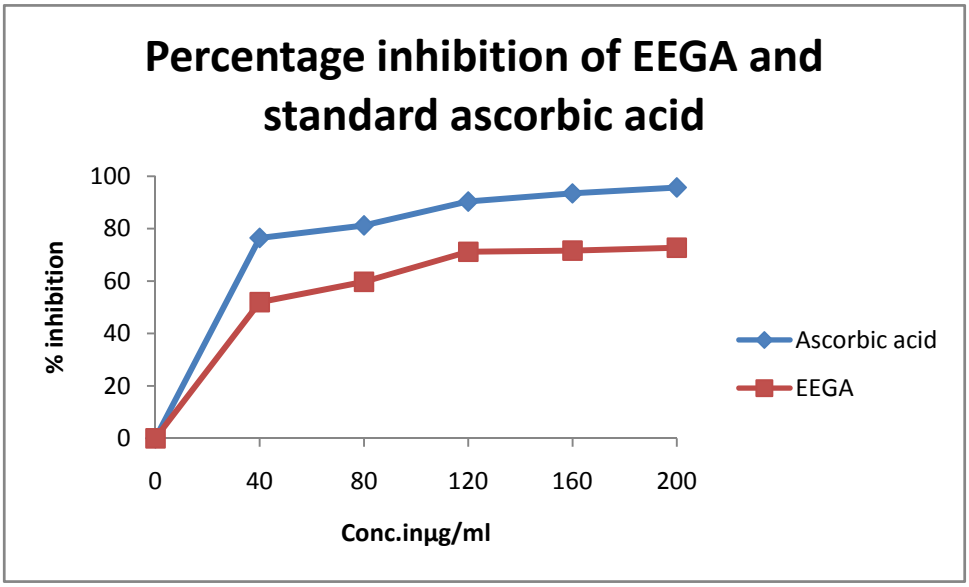
A graph was constructed by plotting concentration versus percentage inhibition and a linear regression equation calculated. The concentration of the sample required for 50% reduction in absorbance (IC_{50}) was calculated using linear regression analysis. A triplicate reading was taken and average was calculated. The results obtained were presented in **Table13**and **Fig.19.**

Table 13: Percentage inhibition by ethanolic extract of *Gmelina arborea* and standard ascorbic acid against DPPH at 517nm

S. No.	Conc. in µg/ml	Percentage inhibition by ascorbic acid	Percentage inhibition by *Gmelina arborea*
1	40	76.44±1.73	51.97±4.66
2	80	81.22±1.13	59.71±4.50
3	120	90.33±2.38	71.18±1.95
4	160	93.45±1.47	71.62±1.87
5	200	95.68±2.02	72.71±2.49
	IC_{50}	**40.68µg/ml**	**85.39µg/ml**

***mean of three readings ± SEM**

Fig. 19: Free radical scavenging assay of ascorbic acid and ethanolic extract of *Gmelina arborea* against DPPH at 517nm



2. **Total antioxidant activity by Phosphomolybdenum Method**

Principle

Total antioxidant capacity was measured by spectrophotometric method of Prieto *et al* [93]. Phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate Mo (V) complex at acidic pH and the absorbance was measured at 695nm. This method is used to determine the total antioxidant activity of samples.

$$Mo^{6+} \rightarrow Mo^{5+}$$

Reagents

0.6M sulphuric acid

28mM sodium phosphate

4mM ammonium molybdate

Instruments

Shimadzu UV Visible spectrophotometer, Model 1800

Procedure

An aliquot of 0.3ml of different concentrations (100,150,200,250,300µg/ml) of sample solutions was combined with 2.7ml of the reagent solution (H_2SO_4, sodium phosphate and ammonium molybdate). In case of blank, 0.3ml of ethanol was used in place of sample. The tubes were incubated for 95°C for 90min. After the mixture was cooled to room temperature, the absorbance was measured at 695nm against blank. The standard Ascorbic acid was treated in a similar manner. The total antioxidant activity is expressed as the number of equivalents of ascorbic acid [94].The results obtained for total antioxidant activity by Phosphomolybdenum method was presented in **Table 14** and **Fig.20.**

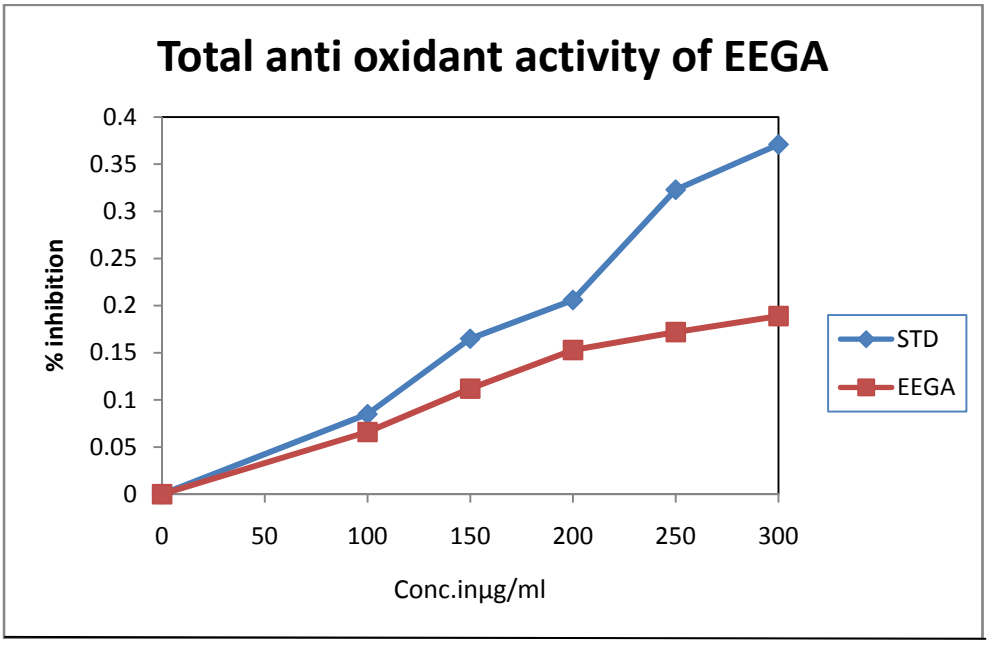
Table14: Total antioxidant activity of ascorbic acid and ethanolic extract of *Gmelina arborea*

S. No	Conc. in µg/ml	Absorbance of ascorbic acid	Conc. in µg/ml	Absorbance of ethanolic extract
1	100	0.085 ± 0.005	100	0.066±0.004
2	150	0.165 ± 0.004	150	0.112±0.013
3	200	0.206 ± 0.008	200	0.153±0.017
4	250	0.323 ± 0.004	250	0.172±0.013
5	300	0.371 ± 0.005	300	0.189±0.004

***mean of three readings ± SEM**

Fig. 20: Total antioxidant activity of

ethanolic extract of *Gmelina arborea*

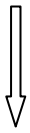


Method 3: Reducing power assay [94, 95]

Principle

Reducing power assay is a spectrophotometric method and is based on the principle that increase in absorbance of the reaction mixture indicates the increase in the reducing power of the sample. Antioxidant activity may be due to a variety of mechanism viz., the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the reductive capacity and free radical scavenging. The assay is based on the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the subsequent formation of prussian blue colour with ferric chloride. The absorbance of the blue complex is measured at 700nm.

Potassium ferricyanide+ Ferric chloride



Antioxidant

Potassium ferrocyanide + Ferrous chloride

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Materials required

Ethanolic extract of *Gmelina arborea leaves*

Ascorbic acid

1% w/v Potassium ferricyanide

10% w/v Trichloro acetic acid

0.2M, Phosphate buffer (pH 6.6)

0.1% w/v Ferric chloride

Procedure

The reducing power ability of plant extracts was screened by assessing the ability of the test extract to reduce $FeCl_3$ solution as mentioned by Oyaizu *et al*., (1986).[95]

0.2 to 1 ml of ethanolic extract of *Gmelina arborea* leaves of (1mg/ml) was mixed with 0.75ml phosphate buffer and 0.75ml of 1% potassium ferricyanide [$K_3Fe(CN_6)$] and incubated at 50^oC for 20min.0.75ml of 1% trichloro acetic acid was added to the mixture ,allowed to stand for 10min. The whole mixture was then centrifuged at 3000rpm for 10min. Finally 1.5ml of the supernatant was removed and mixed with 1.5ml of distilled water and 0.1ml of 0.1%ferric chloride solution and the absorbance measured at 700nm in UV-Visible

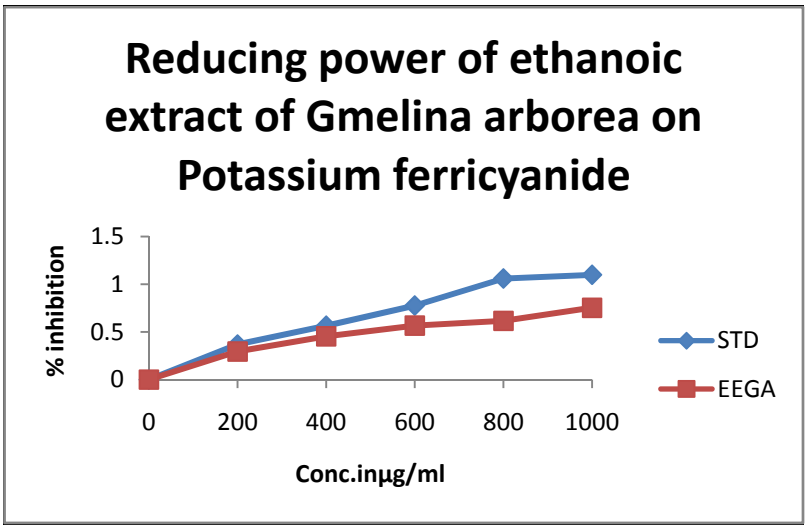
Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used as blank solution. The results obtained were presented in **Table 15** and **Fig.21.**

Table15: Reducing power of ascorbic acid and ethanolic extract of *Gmelina arborea*

S. No	Conc. in µg/ml	Absorbance of ascorbic acid	Conc. in µg/ml	Absorbance of ethanolic extract
1	200	0.3686±0.014	200	0.295±0.029
2	400	0.5650±0.019	400	0.454±0.090
3	600	0.7763±0.033	600	0.566±0.063
4	800	1.0600±0.022	800	0.616±0.054
5	1000	1.0976±0.017	1000	0.753±0.003

***mean of three readings ± SEM**

Fig. 20: Reducing power of ethanolic extract of *Gmelina arborea*



SECTION-B

ANTI -EPILEPTIC ACTIVITY

Epilepsy is the most common neurological disorders affecting people across all nationalities [96]. The word epilepsy is derived from the Greek verb epilamvanein (to be seized", "to be taken hold off", or "to be attacked") indicating that the person having a seizure is 'possessed' or at least out of control [97]. Epilepsy refers to chronic conditions characterized by recurrent seizures [96]. Epilepsy is one of the most common neurological disorders characterized by sudden, transient alterations of brain function usually with motor, sensory autonomic or psychic symptoms often accompanied by loss of, or altered consciousness. Several biochemical hypotheses suggest the involvement of decreased activity of inhibitory GABA ergic system or increased activity of excitatory amino-acids (glutamate and aspartate system) in epilepsy [97].

By electrical stimulation of the brain a variety of seizers can be produced.

The principle being,

a) Supra maximal electrical shock
b) Psychomotor electrical shock
c) Minimal electrical shock

SUPRA MAXIMAL ELECTRO SHOCK (MES) METHOD [98, 99]

The maximal electro-shock (MES) induced convulsions in animals represent grandmal type of epilepsy. This electro-shock assay in rats is used primarily as a indication for compounds which are effective in grandmal epilepsy.

Male albino wistar rats are stimulated through pinna electrodes (150mA current for 0.2sec- stimulus duration)

The resultant seizure in normal rats shows a tonic phase of limb flexion around 2 seconds, followed by full tonic extension phase around 10-13 seconds and a few clonic jerks. Thereafter the numbers of post tonic asphyxia death (or) recovery are noted.

A substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase of MES convulsions.

REQUIREMENTS

Animal : Male albino wistar rats (200-250gm)

Drugs : Standard (Phenytoin sodium)25mg/kg/i.p

Samples : 70% Ethanolic extract of ***Gmelina arborea*** leaves (EEGA)

Instrument : Electro – convulsiometer (Model Ki-9351) Pinna electrodes, stop watch

PROCEDURE

Healthy albino wistar rats weighing from 200 – 220 g were selected. They were kept in separate cages, fed with balanced diet, water and libitum. Then the animals were divided into 5 groups each groups containing six animals.

TREATMENT PROTOCOL

Group-1 Treated as normal control, received 10ml/kg of normal saline through orally.

Group-2 Treated as Standard control, received phenytoin sodium (25 mg/kg) through intraperitonealy.

Group-3 Treated as Treatment Control, received 200mg/Kg of ethanolic extract of *Gmelina arborea* administered through orally

Group-4 Treated as Treatment Control, received 400mg/Kg of ethanolic extract of *Gmelina arborea* administered through orally

All the test compounds were dissolved in 2ml sterile water and administered through orally. The evaluation was started 60 mins after administration of test compounds. Pinna electrodes with the intensity of 150 mA current were used to deliver the stimuli. Inhibition of seizure relative to the control was calculated and the data shown on the **Table 16.**

Table 16:Anti-epileptic activity of EEGA by

Supra maximal electrical shock method

Treatment	Body Wt.	Drug	Dose	Duration of extension phase in seconds	% inhibition of extension phase
Group I	200– 220 gm	Normal saline	10 ml / kg	14.65 ± 2.30	-
Group II	200– 220 gm	Phenytoin sodium	25 mg / kg	2.6 ± 0.65	82.25 %*a
Group III	200– 220 gm	EEGA 200mg/kg	200mg/kg	4.65 ± 0.78	68.25 %*a
Group IV	200– 220 gm	EEGA 400mg/kg	400mg/kg	4.30 ±.0.85	70.64 %*a

- values are expressed as Mean ± SEM
- Values are find out by using One way ANOVA followed by Newman Keul's multiple range tests.
- *aValues were significantly different from normal control (P<0.01)

SECTION-C

IN-VITRO ANTI -ARTHRITIC ACTIVITY BYPROTEIN DENATURATION METHOD

Rheumatoid arthritis (RA) is a chronic inflammatory condition of the connective tissues throughout the body, but especially around the joints. RA is the most common inflammatory arthritis and affects about one percent of the population.[100]It is a common disease having peak incidence in 3rd to 4th decades of life with 3-5times higher preponderance in female.[101]Its prevalence depends upon age. [102]

Rheumatoid arthritis is an autoimmune disorder. One among the cause for the disease is due to the denaturation of the protein. Anti-arthritic activity was studied by inhibition of protein denaturation method.

Instrument

UV/ Visible spectrophotometer at 416 nm.

Materials Required

70% ethanolic extract of leaves of *Gmelina arborea*

Bovine serum albumin (5% w/v aqueous solution)

Phosphate buffer (p^H6.3)

Diclofenac sodium

Procedure [103-106]

Preparation of test solutions

1. **Test solution** (0.5ml)

The test solution consists of 0.45ml bovine serum albumin (5% w/v aqueous solution) and 0.05ml of 70%ethanolic extract of *Gmelina arborea* leaves(200-1000µg/ml concentrations)

2. **Test control solution** (0.5ml)

The test control solution consists of 0.45ml bovine serum albumin and 0.05ml distilled water.

3. **Product control** (0.5ml)

The product control consists of 0.45ml distilled water and 0.05ml of ethanolicextract of *Gmelina arborea* leaves (200-1000 µg/ml concentrations).

4. **Standard solution** (0.5ml)

Standard solution consists of 0.45ml of bovine serum albumin and 0.05ml of diclofenac sodium solution.

All the above test samples were adjusted to P^H6.3 using a small amount of 1N HCl. They were incubated at 37°C for 20 min and heated at 57° C for 3 min and allowed to cool and about 2.5ml of phosphate buffer (p^H 6.3) was added to all the above solutions. The

absorbance was measured by using UV spectrophotometer at 416nm. The percentage inhibition of protein denaturation was calculated using the formula.

$$\text{Percentage inhibition} = 100 - \frac{\{(\text{OD of test solution} - \text{OD of product control})\} \times 100}{\text{OD of test control}}$$

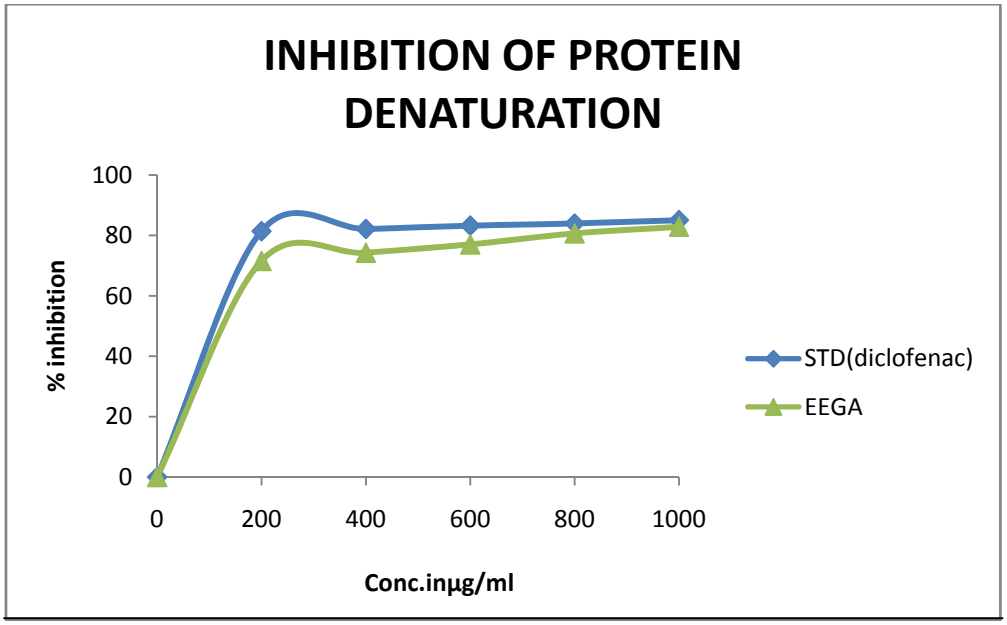
The percentage inhibition of protein denaturation by *Gmelina arborea* was compared with standard diclofenac sodium. The results obtained were presented in **Table 17** and the graphical representation in **Fig.22.**

Table 17: Anti-arthritic activity of EEGA by protein denaturation method

S. No.	Conc. in µg/ml	Percentage inhibition by diclofenac sodium	Percentage inhibition by *Gmelina arborea*
1	200	81.387±8.395	71.533±2.920
2	400	82.116±8.395	74.237±4.5950
3	600	83.212±8.760	77.007±2.555
4	800	83.942±8.760	80.656±0.365
5	1000	85.036±9.125	82.8498±1.097
	IC_{50}	**189.03µg/ml**	**275.71 µg/ml**

***mean of three readings ± SEM**

Fig.22: Effect of diclofenac sodium and *Gmelina arborea* on inhibition of protein denaturation



RESULT AND DISCUSSION

CHAPTER-VIII

RESULTS AND DISCUSSION

This dissertation deals with the "Pharmacognostical, Phytochemical and pharmacological studies of ethanolic extract of *Gmelina arborea* leaves" in an attempt to rationalize its use as a drug of therapeutic importance.

CHAPTER –I

This chapter gives relevant information about the world situation on the traditional medicine and herbal drugs in traditional medicine. Drug discovery from natural products and various strategies for the discovery of drugs from natural resources were discussed in this chapter. Nature and mechanism of epilepsy, classification and medication of epilepsy, mechanism of action of anti-epileptic drugs and causes of rheumatoid arthritis, symptoms, diagnosis and management of rheumatoid arthritis have also been discussed.

CHAPTER-II

In this chapter, the literature review contain the pharmacognostical, phytochemical, pharmacological studies on *Gmelina arborea* and the ethnomedical information about the *Gmelina arborea Roxb* and other related species.

CHPATER-III

In this chapter, aim and scope of the study have been discussed. Pharmacognostical and phytochemical studies which were planned to carry out for this plant were discussed. Pharmacological studies which were planned to carry out for this plant were also discussed in this chapter.

CHPATER-IV

In this chapter scientific classification, common name, synonym, vernacular name and its distribution and the habit of the plant were discussed. Ethnomedical uses were reported.

CHAPTER –V

In this chapter the Macroscopical, Microscopical and physical parameters were discussed.

SECTION-A

Morphological characters of bark,leaves,flower,fruit and root-bark of the plant were described in detail to identify the plant with the support of photographs and drawing as an establishment of authenticity(**Fig 2-6)**

Salient features of the macroscopy of the leaves were observed, such as the leaves are simple, opposite- decussate, broadly ovate, 10-25cm long and 7-20cm wide, apically long acuminate apex or caudate, entire on mature plants but strongly toothed or lobed on young plants. Flowers appear with or sometimes before the young leaves.

SECTION-B

This section deals with the microscopical studies of the leaves to ascertain the arrangement of tissues.**(Fig.7-10)**

The following features have been observed in the transverse section of the leaves of *Gmelina arborea.*

- The leaf consists of a thick midrib and thin lamina with densely tomentose abaxial surface.

- The Lamina consist of thick and wide, vertically oblong epidermal cells with prominent cuticle.
- The stomata are actinocytic type.
- The epidermis bears dense non glandular trichomes.
- The mesophyll cells consist of adaxial palisade and abaxial spongy parenchyma cells.
- The palisade cells are single layered with thin vertical, long cylindrical cells and cylindrical, spherical or lobed parenchyma cells in spongy mesophyll tissue.
- The vascular strand consists of two arc shaped collateral bundles.
- The venation is densely reticulate. The primary, secondary and tertiary veins are thin, but distinct .The vein- islets are wide and well defined with prominent vein – boundaries. The vein- terminations, when present are long, slender and forked at the tip.

SECTION – C

It deals with the quantitative microscopy such as stomatal number, stomatal index, vein - islet number, vein termination number which were determined and tabulated as follows.

Quantitative analytical microscopical
Parameters of the leaf of Gmelina *arborea*

S. No.	Parameters*	Values obtained*
1.	Stomatal number in lower epidermis	41.33±0.66
2.	Stomatal index in lower epidermis	27.33±0.33
3.	Vein islet number	35.67±0.88
4.	Vein termination number	19±0.58

*** mean of 3 readings ± SEM**

SECTION-D

Physical parameters:

The following physical parameters like ash values, loss on drying, extractive values, foaming index were determined and tabulated.

Ash values and loss on drying

	Total ash	Acid insoluble ash	Water soluble ash	Sulphated ash	Loss on drying
Minimum	5	0.16	3.4	9.03	8.5
Average	7.8	0.23	3.6	9.16	9.0
Maximum	9.5	0.33	3.9	9.22	9.6

Extractive values by cold maceration

S.NO	SOLVENTS USED	EXTRACTIVE VALUE (%W/W)
1	Petroleum ether	30.07
2	Ethyl acetate	6.55
3	Chloroform	4.87
4	Ethanol	11.31
5	Acetone	9.28
6	Water	26.38

SECTION-E

A.Fluorescence analysis

Extract	Day light	UV light (254nm)	UV light (366nm)
Pet. Ether	Light brown	Light green	Green
Ethyl acetate	Green	Dark green	Orange
Aqueous	Light brown	light green	Green
Chloroform	Brown	Brownish green	Orange
Acetone	Green	Dark green	Orange
Ethanol	Green	Dark green	Orange

B.Powder microscopy

The following anatomical characters were observed with powder microscopy of leaves reveals the presence of

- Multicellular-uniseriate unbranched epidermal trichomes
- Fragments of spongy parenchyma
- Fragments of palisade cells
- Epidermal cells with actinocytic stomata
- Spiral xylem vessel

CHAPTER –VI

Phytochemical studies

SECTION-A

This deals with the organoleptic evaluation of the leaves of *Gmelina arborea Roxb.*

SECTION-B

Preliminary phytochemical screening:

This chapter was deals with the phytochemical studies of the leaves of *Gmelina arborea*. The results obtained for the preliminary phytochemical screening of the various extracts of plant material were presented in **Tables 6** and **7.**From the table, it can be observed that the ethanolic extract of Gmelina arborea leaves showed the presence of carbohydrates, saponins, alkaloids, flavonoids, Tannin and phenolic compounds while the petroleum ether and chloroform extracts showed the presence of sterols. The aqueous extract showed the presence of carbohydrate, protein, saponins and tannin.

SECTION-C

1. Determination of total phenolic content

The total phenolic content for the ethanolic extract of *Gmelina arborea* leaves was found to be 74.43±4.825mg/gm respectively, and shown in **Table 8.** Phenolic substances are known to possess the ability to reduce oxidative damage and act as antioxidants.

2. Determination of total flavonoid content

The total flavonoid content for the ethanolic extract of *Gmelina arborea* leaves was found to be 51.32±4.82mg/gm respectively, and shown in T**able 9.**

3. Determination of total tannin content

The total tannin content for the ethanolic extract of *Gmelina arborea* leaves was found to be 33.23±2.25mg/gm respectively, and shown in **Table 10.**

These substances associated with antioxidant activity and played important role in stabilizing lipid per-oxidation.

SECTION-D

TLC and HPTLC STUDIES

1. TLC

Thin layer chromatography studies were carried out for the ethanolic extract of Gmelina arborea using Ethyl acetate: Benzene and Chloroform: Ethyl acetate as solvent systems and the reports were presented in the **Table.11.**

Table 11: TLC of the ethanolic extract of *Gmelina arborea*

S. No	Solvent system	Detecting agent	No of spots	Colour of spots	$R_f$values
1.	Ethyl acetate: Benzene (2:8)	Under UVlight at 365nm	I	Yellow	0.37
			II	Orange fluorescence	0.8
			III	Yellow	0.92
2.	Chloroform:Ethylactate (60:40)	Under UVlight at 365nm	I	Yellow	0.23
			II	Orange fluorescence	0.8
			III	Yellow	0.92

2. HPTLC

The HPTLC chromatogram for the ethanolic extract of *Gmelina arborea* leaves showed 8 peaks at 254nm and the R_f values were found to be 0.11, 0.16, 0.21, 0.44, 0.49, 0.61, 0.82, 0.90.Nine peaks at 366nm and the R_f values were found to be 0.08, 0.16, 0.23, 0.36, 0.45, 0.49, 0.59, 0.66, 0.7.The HPTLC finger print profile of the ethanolic extract of

Gmelina arborea showed various peaks having different $R_f$values indicates that the plant contains many medicinally active compounds which may be responsible for its therapeutic activity.

CHAPTER-VII

SECTION-A

In-vitro anti - oxidant activity

Method 1: Free radical Scavenging activity using diphenylpicrylhydrazyl (DPPH) free radical

From the results obtained it has been observed that the ethanolic extract of *Gmelina arborea* leaves showed a percentage inhibition of 72.71%while the standard, ascorbic acid showed a percentage inhibition of 95.68%at a concentration of 200µg/ml (showed in **Fig.19** and **Table 13**). The IC_{50}value was found to be 85.39µg/ml and 40.68µg/ml for the ethanolic extract of *Gmelina arborea* leaves and ascorbic acid respectively. The extract possessed a good radical scavenging capacity.

Method 2: Total antioxidant activity by Phosphomolybdenum Method

It is observed that the ethanolic extract of *Gmelina arborea* leaves showed an absorbance of 0.189±0.004for a concentration of 300µg/ml while ascorbic acid showed an absorbance 0.371±0.005 at a concentration of 300µg/ml.(showed in **Fig.20** and **Table 14**.

Method 3: Reducing power assay

It is observed that the ethanolic extract of *Gmelina arborea* showed an absorbance of 0.753±0.003 for a concentration of 1mg/ml while ascorbic acid showed an absorbance 1.0976±0.017 at a concentration of 1mg/ml. (showed in **Fig.21** and **Table 15**).

The ethanolic extract of *Gmelina arborea* leaves, which contains large amount of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. It also chelates iron and has reducing power. These in-vitro assays indicate that this plant extract is a

significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

SECTION-B

ANTI-EPILEPTIC ACTIVITY

This study deals with the in-vivo pharmacological screening of anti-epileptic activity of *Gmelina arborea* by supra maximal electro shock method. From the results obtained, it has been observed that, the percentage inhibition of seizures for the ethanolic extract of Gmelina *arborea* leaves was found to be68.25% and70.64%at a dose of 200mg/kg and 400mg/kg. The standard phenytoin sodium shows 82.25% at a dose of 25mg/Kg **(showed in Table.16)**. This study reveals that the ethanolic extract of *Gmelina arborea* leaves possesses significant anti-epileptic activity ($P<0.01$). The extract was reducing the duration of extensor phase significantly when compared to normal control.

SECTION-C

IN-VITRO ANTI-ARTHRITIC ACTIVITY BY PROTEIN DENATURATION METHOD

Denaturation of protein is one of the causes of rheumatoid arthritis. The production of auto antigens in certain rheumatic diseases may be due to protein denaturation which involves the alteration of electrostatic hydrogen, hydrophobic and disulphidebonding [107].The protein used in this study is bovine serum albumin. Denaturation of protein is carried out by heating. The aim of this activity is to inhibit protein denaturation and to exhibit protective effect against rheumatoid arthritis.

From the results obtained, it has been observed that, the percentage inhibition of albumin denaturation by ethanolic extract of *Gmelina arborea* leaves was found to be 82.85% and for standard diclofenac sodium 85.04% at a concentration of 1mg/ml (showed in the **Fig**

.22and **Table 17**).The IC_{50}value was found to be 275.71 µg/mland189.03µg/ml for ethanolic extract of *Gmelina arborea* leaves and diclofenac sodium respectively. This study showed that the extract has a significant anti-arthritic activity.

The extract showed good antioxidant properties which may be due to the presence of phenolic compounds and flavonoids in the extract. A very good in-vitro protein denaturation effect showed that the plant extract has significant anti-arthritic activity.

SUMMARY AND CONCLUSION

CHAPTER-IX

SUMMARY AND CONCLUSION

This dissertation entitled "Pharmacognostical, Phytochemical, and Pharmacological evaluation on the leaves of *Gmelina arborea Roxb. (*Verbenaceae).The macroscopical, microscopical characters, powder analysis, quantitative microscopy and standardization parameters like ash value, extractive value, loss on drying and fluorescence have been studied and presented.

The gist of the findings derived from the investigation under this project is furnished in the following concluding lines.

Pharmacognostical parameters have been determined for the leaf of *Gmelina arborea Roxb* in order to substantiate and identify the plant for future work.

The kinds of microstructures have been recognized as tools to measure the phylogenetic relationship under light microscope, to resolve taxonomic controversies and to establish the botanical identity of the plant.

Preliminary phytochemical screening of the leaf of *Gmelina arborea Roxb* reveals the presence of sterols, carbohydrates, protein, alkaloids, flavonoids, tannin and mucilage.

Determination of tannins, phenolic content and flavonoid content confirms that significant concentrations of these phytoconstituents are present in the ethanolic extract of *Gmelina arborea Roxb*.

TLC studies were performed with the ethanolic extract of the leaf to identify the phytoconstituents present in the leaf of this plant.

HPTLC finger print studies were performed to evaluate the active constituent present in the leaf of *Gmelina arborea Roxb*.

Pharmacological screening confirms that the ethanolic extract of *Gmelina arborea* leaves showed significant antioxidant activity which was evaluated by

Free radical scavenging by DPPH assay

Total antioxidant activity

Reducing power assay

The ethanolic extract of *Gmelina arborea* leaves showed significant anti-epileptic activity which was evaluated by **MES method.**

The result of the anti-epileptic activity study clearly indicate that the ethanolic extract of *Gmelina arborea* leaves has significant anti-epileptic activity as assessed by the reduction in time of tonic extensor phase of MES TEST as shown in **Table 15**.

The result of the MES TEST (**Table 15**) reveals that the ethanolic extract possesses significant anti-epileptic activity ($P<0.01$). The extract was reducing the duration of extensor phase significantly when compared to normal control.

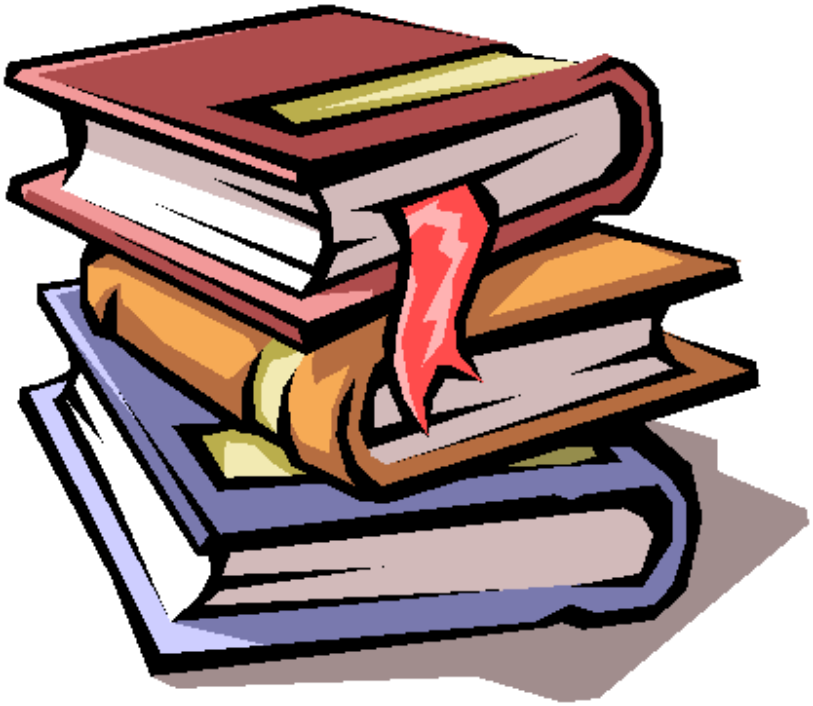
So we can conclude that the ethanolic extract of *Gmelina arborea* leaves had anti-epileptic activity .However it is difficult at the moment to indicate the exact nature and category of such actions of the EEGA which require more investigation before any definite conclusion can be drawn in this aspect.

The ethanolic extract of *Gmelina arborea* leaves showed significant anti-arthritic activity which was evaluated by **Protein denaturation method by using bovine serum.**

The extract showed more anti-arthritic activity compared to standard diclofenac sodium. The activities may be due to the presence of tannins, flavonoids and alkaloids.

The present study provides scientific basis for the folkloric medicinal application of the leaves of *Gmelina arborea* for the treatment of rheumatoid arthritis and epilepsy.

The future scope of the work is to isolate the chemical constituents responsible for the activities and also to carry out pharmacological screening in vivo models.



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Thank
You