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PHARMACOGNOSTICAL, PHYTOCHEMICAL AND DIURETIC ACTIVITY OF TAMILNADIA ULIGNOSA (Retz.) Tirven&Sastre (RUBIACEAE)

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Abstract : Many of the synthetic drugs currently used for the treatment of diseases are not only expensive but also having several side effects. So, this situation has forced the scientists to seek alternative drugs.Many plant drugs have been traditionally used as Diuretic. The plant *TamilnadiaUlignosa*(Retz.) Tirven&Sastre belonging to the family Rubiaceae, medicinallay it is used as diuretic.But no evidence is available for the scientific research of the plant for the said activity. The plant will be studied for the same. A suitable plant extract and evaluated for the physico-chemical parameters and pharmacological activity.

IndexTerms - Indian System of Medicine,Research and Development,International Conference of Drug Regulatory Authorities,Dietary Supplement and Health and Education Act,Indian Pharmacopoeia,Ascending Loop of Henle,Distal Convuluted Tubules.

1.INTRODUCTION

Pharmacognosy is the objective study of crude drugs of animal, vegetable and mineral origin treated scientifically.

Indian System of Medicine (ISM) has been introduced from time immemorial in the traditional practice to treat various ailments and now it's becoming globally accepted with scientific evaluation due to their curative properties (Soumyaprakash etal.,2009)

Herbal medicines sometimes referred to as Herbalism or Botanical Medicine is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities (web 1). The world health organization defines 4 types of herbal medicines. They are as follows

- Raw herbs
- Herbal materials: plants juices, oils, resins, dry powders.
- Herbal preparations: herbal extracts and tinctures of herbal materials produced by biological/chemical methods such as extraction, fractionation, purification and concentration. It is the basis for finished herbal products.
- Finished herbal products: May contain inactive compounds that facilitate dilution or delivery of active ingredient in herbal preparation. May contain single or multiple ingredients. Some may include natural ingredients not of plant origin, such as animal products or minerals. (web 2)

Herbal medicines which formed the basis for health care throughout the world since the earliest days of mankind and it is still the main stay of about 75-80% of world's population mainly in developing countries, for primary health care because of better cultural

acceptability, better compatibility with the human body and lesser side effects. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plant substances. Aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine and vinblastine are a few important examples of what medicinal plants have given us in the past. Most of these plant-derived drugs were originally discovered through the study of traditional cures and folk knowledge of indigenous people and some of these could not be substituted despite the enormous advancement in synthetic chemistry. Consequently, plants can be described as a major source of medicines, not only as isolated active principles to be dispensed in standardized dosage form but also as crude drugs for the population.

Today in many countries modern medicine has displaced plants with many synthetic products but almost 30% of pharmaceutical preparations are still obtained directly or indirectly from plants. The modern era has seen some decline in use of medicinal plants and their extracts as therapeutic agent, particularly in developed countries, many of which either been discarded by the medical profession or now given in the form of isolated compound.

The strategy of isolating the active principles from the medicinal plants and manufacturing a pharmaceutical preparation then became popular. Modern medicines and herbal medicines are complimentarily being used in areas for health care program in several developing countries including India. Of late, the interest in the plant products surfaces all over the world due to the belief that many herbal medicines are known to be free from side effects.

It is the fact that the discovery of the new synthetic drug is time consuming & an expensive affair. The utility of the synthetic drug is always accompanied with its single or multiple adverse effects and in some cases the curatives are not available. Herbs had been used by all cultures throughout history but India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value.

In many journals, national and international, increasing number of research publications based on herbal drugs, plants have provided mankind a large variety of potent drugs to alleviate suffering from diseases in spite of spectacular advances in synthetic drugs in recent years; some of the drugs of plant origin have still retained their importance. The use of plant-based drugs all over world is increasing. Inspite of the tremendous advances made in the modern medicine there are still a large number of ailments for which suitable drugs are yet to be found. Today, there is an urgent need to develop safer drugs for the treatment of inflammatory disorders, diabetes, liver diseases, and gastrointestinal disorder. Hence, there is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine (web 3).

Herbal medicine is the use of plants (herbs) to treat disease or state of wellbeing. Herbal medicine is used to treat a range of disorders including anxiety, arthritis, depression, high blood pressure, insomnia, hormonal imbalance, migraines skin problems such as eczema and other disorders. Herbs can act on the body as powerful as pharmaceutical drugs.

Herbal medicine has its origin in ancient culture including those of Egyptians, Americans, Indians and Chinese. It involves the medicinal use of plants to treat disease and enhance general health and wellbeing.

In recent years interest in herbal medicine has sky rocketed, leading to a greater scientific interest in the medicinal use of plants. Many international studies have shown that plants are capable of treating the disease and improving health, often without any significant side effects. (web 4)

1.1. Traditional Medicines Importance

Traditional medicine is the sum total of the knowledge, skill, and practices based on theories, beliefs, and experiences Indigenous to different culture, used in the maintenance of health as well as in the prevention, diagnosis, improvement or development of physical and mental illness. The terms complementary medicines or alternative medicines are used interchangeably with traditional medicine in some countries. They refer to a broad set of health care practices that are not the part of countries own tradition and not integrated in to dominant health care system. (Web 4)

WHO recognized the importance of herbal medicines to the health of many people throughout the world, stating: A few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore, or improve health (web 5).

In many countries, 80% or more of the population living in rural areas are cured by traditional medicine practitioners and birth attendants**.** It has since revised its view**,** adopting a rather safer position, now stating: most of the populations of most developing countries regularly use traditional medicine; only a minority has regular access to reliable modern medicinal service. (Web 6)

Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities. (Web 4)

Traditional medicine is perceived as efficient safe and cost effective. Moreover, it is accessible to the poor and those living in remote areas. In view of this broad appeal, the general lack of research on the safety and efficacy of traditional medicines is of great concern.

1.2. Role of Medicinal Plants

Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India.

There are about 45,000 plant species in India, with concentrated spots in the region of eastern Himalaya, Western Ghats, Andaman and Nicobar Island. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. India is the largest producer of medicinal herbs and is called the botanical garden of the world (web 7).

Some of examples of medicinal plants which shows the medicinal benefits are

- Ginkgo (*Ginkgo biloba*) has been used in traditional medicine to treat circulatory disorders and enhance memory.
- Kava kava (*Piper methysticum*) is said to elevate mood, enhance well being and contentment, produces the feeling of relaxation. It is used in the treatment of anxiety, insomnia and nervous disorders.
- Saw palmetto (*Serenoarepens)*is used for the treatment of benign prostatic hyperplasia (BPH), a non cancerous enlargement of the prostate gland. The herb is effective for treating frequent urination.
- St. John's wort (*Hypericumperforatum*) is well known for its antidepressant effects. And in the treatment of mild and moderate depression.
- Valerian (*Valerianaofficinalis)* is a popular alternative to commonly prescribed medications for sleep problems because it is considered to be both safe and gentle. Some studies bear this out, although not all have found valerian to be effective.
- Echinacea preparations (from *Echinacea purpurea*andother*Echinacea*species) may improve the body's natural immunity (web 8).

1.3. Difference of Herbal and Conventional Drugs

Although superficially similar, herbal medicine and conventional pharmacotherapy have three important differences:

Use of Whole Plants- Herbalists generally use unpurified plant extracts containing several different constituents. It is claimed that these can work together synergistically so that the effect of the whole herb is greater than the summed effects of its components. It is also claimed that toxicity is reduced when whole herbs are used instead of isolated active ingredients ("buffering"). Although two samples of a particular herbal drug may contain constituent compounds in different proportions, practitioners claim that this does not generally cause clinical problems. There is some experimental evidence for synergy and buffering in certain whole plant preparations, but how far this is applicable toall herbal products is not known.

Herb Combining- Often several different herbs are used together. Practitioners say that the principles of synergy and buffering apply to combinations of plants and claim that combining herbs improves efficacy and reduces adverse effect. This contrasts with conventional practice, where polypharmacy is generally avoided whenever possible.

Diagnosis-Herbal practitioners use different diagnostic principles from conventional practitioners. For example, when treating arthritis, they might observe, "Under functioning of a patient's symptoms of elimination" and decide that the arthritis results from "an accumulation of metabolic waste products". Adiuretic or laxative combination of herbs might then be prescribed alongside herbs with anti-inflammatory properties (Sanjoy Kumar pal.*et al.*,2003).

1.4. Regulation and Registration of Herbal Medicine

The legal situation regarding herbal preparations varies from country to country. In some, phytomedicinesarewell-established, whereas in others they are regarded as food and therapeutic claims are not allowed.Developing countries, however, often have a great number of traditionally used herbal medicines and muchfolk-knowledge about them, but have hardly any legislative criteria to establish these traditionally used herbalmedicines as part of the drug legislation.

For the classification of herbal or traditional medicinal products, factors applied in regulatory systems include, description in a pharmacopoeia monograph, prescription status, claim of a therapeutic effect, scheduled orregulated ingredients or substances, or periods of use. Some countries draw a distinction between "officiallyapproved" products and "officially recognized" products, by which the latter products can be marketed without scientific assessment by the authority.

The various legislative approaches for herbal medicines fall into one or other of the following categories:

- Same regulatory requirements for all products.
- Same regulatory requirements for all products, with certain types of evidence not required for herbal/traditional medicines.

1.5. Present Status of Herbal Medicine

The wide spread use of herbal medicine is not restrictedto developing countries, as it has been estimated that 70%of all medical doctors in France and German regularlyprescribe herbal medicine. Thenumber of patients seeking herbal approaches for therapy isalso growing exponentially. Withthe US Food & Drug Administration (FDA) relaxingguidelines for the sale of herbal supplement,the market is booming with herbal products.

As per the available records, the herbal medicine market in1991 in the countries of the European Union was about $ 6billion (may be over $20 billion now), with Germany accountfor $3 billion, France $ 1.6 billion and Italy $ 0.6 billion. In1996, the US herbal medicine market was about $ 4 billion,which have doubled by now.

The Indian herbal drug marketis about $ one billion and the export of herbal crude extractis about $80 million.In the last few decades, a curious thing has happened tobotanical medicine. Instead of being killed off by medicalscience and pharmaceutical chemistry, it has made comeback.

Herbal medicine has benefited from the objectiveanalysis of the medical science, while fanciful and emotionalclaims for herbal cures have been thrown out, herbaltreatments and plant medicine that works have beenacknowledge. And herbal medicine has been found to havesome impressive credentials. Developed empirically by trialand error, many herbal treatments were neverthelessremarkably effective.

2. REVIEW OF LITERATURE OF TAMILNADIA ULIGNOSA (Retz.) Tirven&Sastre(Rubiaceae)

2.1. Ethnobotanical Review

Botanical Name: *Tamilnadiauliginosa*(Retz.) Tirven&Sastre

Family: Rubiaceae

Synonyms: *Gardenia uliginosa, Catunaregamuliginosa, Randiauliginosa, Gardenia pomifera, Catunaregamuliginosa*(Retz)*Manilal&Sivar, Xeromphisuliginosa*(Retz.) Mahesh W (Web 10)

Vernacular names

English : Divine jasmine, Tamilnadia, Grey emetic nut,

Telugu : Adivimanga, Peddamanga, Devatamalle

Sanskrit : Devathamalla (web 10,sudhakar k.*et al.*, 2012)

Taxonomy:

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Magnoliatae

Order : Rubiales

Family : Rubiaceae

Genus : Tamilnadia

Species : *Uliginosa*(web 10)

Fig No.1.Tamilnadia Ulignosa(Retz.) Tirven&Sastre(Rubiaceae)

Occurrence and Distribution (Sudhakar K *et al.*, 2012)**:**

The plant is grown in all dry districts in open forests, native to Bangladesh, India, Sri Lanka, Thailand, and Vietnam. The plant is grown at an altitude of 1000m common in the sub Himalayan tracts from the Yamuna eastwards and in eastern central and southern India. In Andhra Pradesh it is available in all districts.

Botanical description:

Habit: *Tamilnadiauliginosa*, is a very rigid, ramous, dry deciduous,small armed tree with quadrangular branches up to 7.5m height and it is widely distributed in India, Srilanka, Bangladesh, Thailand, Vietnam.

Branches: Branches are erect rigid, quadrangular, thick set with short, rigid round, diverging branchlets. Short lateral shoots, each of which terminally produces one or two pairs of short thorns.

Leaves: Leaves opposite on young shoots, or fascicled at the end of branch lets, short-petioled, oblong, shining, entire, 2-3 inches long by 1.5 inch broad.

Flowers: Flowers are dimorphic, white and fragrant. Usually the tree flowers in months between April to June

Berries: Berry ellipsoid or ovoid solitary 5-6cm long, yellowish brown, crowned with persistent calyx; stalk 2 cm long; seeds are compressed, smooth, closely packed in pulp.

Fruits: fruits are edible and it is ripen in February to march

Root: The root is whitish grey or light-brown, closegrained, and hard.

2.2. Ethnomedicinal Review: (Sudhakar K *et al.*, 2012)

Venkata RatnamK. *et al.*,(2008) conducted a survey in adivasis of Eastern Ghats, Andhra Pradesh. The local name of the plant is adavijama. Stem bark of *Tamilnadiauliginosa*was used for bone fractures ground with white layer of country egg, turmeric and calcium.

PrustiA.B. *et al.*, (2007) conducted a ethnobotanical survey in Malkangiri District of Orissa, this survey describes that koyaguda local people named tamilnadiauliginosa as kumudmara, they used raw fruits as vegetable.

NarayananM.K.R.*et al.*, (2011)conducted a survey in the Wayanad Wildlife Sanctuary on floristic and ethnobotanical studies; locally they called as Pindichakka and found that tender fruits are used as vegetables.

ReddyK N. *et al.*, (2000-2005) conductedanEthno pharmacological survey in Chittoor,Cuddapah, East Godavari, Guntur, Khammam, Krishna, Kurnool, Srikakulam, Visakhapatnam, Vijayanagaram and West Godavari districts of the rural people and forest ethnic people (Chenchus,Erukulas, Lambadas, Koyas, Kondareddies,Nukadoras, Yanadis). They found that the stem bark of Helicteresisora Linn along with that of *Tamilnadiauliginosa* and a whole plant of Bacopa monnieriWettst (10 gm. each) are used in treatment of Cold and Cough.

WongsatitChuakul*et al.*, (2002) conducted asurvey on medicinal plants in Kutchum District, Yasothon Province, Thailand. The decoction of root of *Tamilnadiauliginosa*was used in treatment of diabetes mellitus.

Table1: Traditional uses and local names of *Tamilnadiauliginosa*(Retz)Triveg&Sastre

S. No	Region	Local name	Plant part used	Medicinal uses	References
1	Koyaguda	Kumudmara	Fruits	Astringent,Colour Intensifier	Sudhakar K.*et al.*, (2012)
2	Eastern ghats of A.P.	Adivijama	Stem bark along with egg,turmeric and calcium	To treat bone fractures	Sudhakar K.*et al.*, (2012)
3	Wayanad wild life sanctuary, kerela	Pindichakka	Fruits	Used as vegetables	Sudhakar K.*et al.*, (2012)
4	Thailand	Mui khao-talumphuk	wood	To treat Diabetes mellitus	WongsatitChuakul et al.,(2002)
5	Chittoor	Adavimanga	Root	Used as Diuretic&To treat Hypertension Dysentry	Dr. K.;Madhava Chetty, Asst. Professor, S.V. University

2.3. Pharmacological Review:

Antidiarrhoeal activity:

Anti-diarrhoeal effects of ethanolic (90%) extract of dried fruits of *Tamilnadiauliginosa*(Retz.) Tirven&Sastre(METU) was studied against castor oil-induced-diarrhoea model in rats. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method. Standard drug atropine (3mg/kg, i.p) showed significant reductions in fecal output and frequency of droppings whereas METU at the doses of 100 and 200 mg/kg i.p significantly retarded the castor-oil induced enteropooling and intestinal transit. The gastrointestinal transit rate was expressed as the percentage of the longest distance travelled by the charcoal divided by the total length of the small intestine. METU at the doses of 100 and

200 mg/kg significantly inhibited (P<0.001) weight and volume of intestinal content. The results obtained establish the efficacy and substantiate the folklore claim as an anti- diarrheal agent. **(**Zechariah Jebakumar A. *et al.*,**)**

2.4 Antimicrobial Activity

India has rich plant diversity. The people in India are using these plants for medicinal purposes. This practice is common in other developing countries also. The drugs obtained from Different of plants are used in various traditional as well as modern Practices. The present investigation is aimed at investigating the in vitro Successive solvent extract viz., petroleum ether, chloroform, methanol, and water extracts of stem bark of Tamilnadiauliginosa was evaluated for antibacterial activity, against four important bacterial strains S. aureus Escherichia coli, Lacto bacillus, and Enterococcus fecalis by agar-well diffusion method. All the solvent extract showed significant activity against all the tested bacteria, The antibacterial activity is more significant in solvent extracts compared to aqueous extract indicating that the active principle responsible for antibacterial activity is more soluble in organic solvents Comparison of the inhibitory activity of the extracts with the antibiotics Gentamicin revealed that methanol extracts of Tamilnadiauliginosa was significantly higher than that of the antibiotics tested. The results suggest that Tamilnadiauliginosa. Can be used as a medicament for oral infection.(G. Sandhya Rani 1,Bikku Naik 1, Alli Ramesh 2, Balaji B2).

3.PHARMACOGNOSTICAL STUDIES OF THE ROOTS OFTAMILNADIA ULIGNOSA (Retz.) Tirven&Sastre (Rubiaceae)

Introduction

Macroscopical, microscopical and inorganic constituents present in a drug or plant play a significant role in identification of crude drug. Macroscopical and microscopical characters will help in the identification of right variety and search for adulterants. Physical constants like ash and extractive values help in establishing the pharmacopoeial standards of drug. Fluorescence analysis help to identify the drug in powder form. Physical constants were determined following Kokate (1999) and *Indian Pharmacopoeia* (1996).

Collection and Authentication

The rootof*Tamilnadiauliginosa (*Retz) Tirven&Sastre (Rubiaceae)were collected from Tirumala hills, Tirupathi, India in the month of October 2013 and it was identified and authenticated. The taxonomical identification and authentication of the plant was done by **Dr. K. Madhavachetty**, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati. The voucher number is 1544 and specimen was preserved in our laboratory for further reference.

6.3Macroscopic Studies

It is assumed thatmacroscopical evaluation of any plant drug is considered to be the primary step for establishing its quality control profile. Proper authentication of a drug depends almostentirely on macroscopical description of a crude drug includes size, nature of outer and inner surfaces, type of fracture and organoleptic characteristics like color, odour, taste etc.

Fig No. 2. Roots of TamilnadiaUlignosa

6.4Microscopic Studies

METHOD

The fresh sample were cut into small pieces and fixed in FAA solutions (Formalin 5ml + Glacial acetic acid 5ml + 70% methanol 90ml). After fixing the specimens were dehydrated with grated series of tertiary butyl alcohol (TBA) as per the standard procedure (Sass, 1940). After complete dehydration, the specimens were embedded in paraffin wax. The paraffin embedded specimens were sectioned with the help of Rotary microtome (thickness 10-12µm). Dewaxing and staining of the sections were done by customary procedure (Johansen, 1940).

STAINING

1. Cholral hydrate solution + Phloroglucinol + Conc HCLs

Photomicrograph

All permanent slides, after staining were dehydrated by using graded series of Methanol + Fibers and Calcium Oxalate crystals in DPX. Photomicrographs were done on NIKON – Olympus – 2 microscope using Konica colour film (100 ASA). For normal observations bright filed was used. For the study of crystals and starch grains, the sections were photographed under polarized light. Magnifications of the figures are indicated by scale bars.

Descriptive terms of various observations are as found in standard anatomy books(Easau,1979).

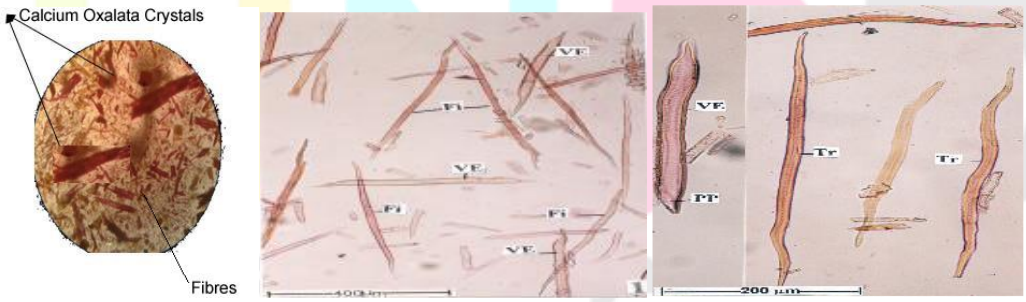


Fig . Powder microscopy of *Tamilnadiauliginosa*
VE-Vesselelement;

Fb-Fibre,

COC- Calcium Oxalate crystals

Presence of Fibers, Calcium Oxalate Crystals in the powder is a characteristic diagnosing future of TamilnadiaUlignosa.

6.5Physico-Chemical Parameters

6.5.1 Determination of moisture content

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Drying plays a very important role in the quality as well as purity of the material. Moisture will lead to the activation of enzymes and gives suitable condition, to the proliferation of microorganisms. (Practical Pharmacognosy text book by Dr.B.Durai Swamy and Prof. K.Jayaveera, S.Chand).

Method

About 2 g of the drug was weighed in a watch glass, kept in hot air oven at 105^0C and dried for a period until constant weight was obtained. Weight loss on drying was noted and difference in weight gives the moisture content of powdered drug. Total moisture content of root was note.

6.5.2 Determination of ash value

Ash value aids in determination of quality and purity of crude drug in powdered form. The ash content of a crude drug is generally considered as a residue remaining after maceration. Ash contains inorganic salts like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium are adhere to it or may also be added to for the purpose of adulteration. There is a considerable difference (varies with in narrow limits) in the case of same individual drug. Hence ash determination furnishes a basis for judging the identity and quality of the drug gives information to its adulteration with inorganic matter. Ash standards have been established for a number of drugs in the pharmacopoeias. The acid insoluble ash is a part of ash is imposed, especially in case where silica and calcium oxalate content of the drug is very high. In most of the cases inorganic matter is present in small amounts which are not objectionable if only traces are present. Procedure is given in Indian pharmacopoeia were used to determine the different ash values such as total ash, acid insoluble ash.

6.5.3 Determination of total Ash value

Weigh accurately 3gms of the powdered material in a silica crucible which was previously ignited and weighed. The powdered material was spread as a fine even layer at the bottom of the crucible. The crucible was incinerated until a red hot material was obtained not exceeding 450°C temperature and it is free from carbon. The crucible was cooled and weighed. The procedure was repeated until the constant weights. The percentage of the total ash was calculated with reference to the air dried powdered sample.

6.5.4 Determination of acid insoluble ash value

The obtained total ash was boiled with 25 ml of 2N Hcl for 5 min. The insoluble ash was collected on ash less filter and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible, ignited, cold and weighed. The procedure was repeated till the constant weight was obtained. The percentage of acid insoluble ash was calculated with reference to the air dried drugs.

6.5.5 Determination of water soluble ash value

The total ash obtained was boiled with 25 ml of chloroform water for five min. The insoluble matter was collected on a ash less filter paper & and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible, ignited for 15 min at a temperature not exceeding 450 ^{0}c, cooled and weighed. The procedure was repeated to get the constant weight. The weight of the insoluble matter was subtracted from the weight of total ash. The percentage of water soluble ash was calculated with

reference to the air-dried sample drug.

Table 2. Physicochemical parameters of powdered *Tamilnadiauliginosa*

S.No	Parameters	Average % w/w
1.	**Ash values**	
	a)Total ash	18
	b)Acid insoluble ash	8.4
	c)Water insoluble ash	4.1
2.	**Moisture content**	7.5

6.6Fluorescence Analysis

Fluorescence provided by a drug is one of the several methods used for analyzing crude drugs. Fluorescence is a type of luminescence in which the molecule emits visible radiation passing from higher to lower electronic state. The molecules absorbs light usually over a specific range of wavelength, get excited from ground state to a high energy level and many of them emit such radiations while coming back to the ground state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "Fluorescence". For fluorescence analysis, powdered drug was sieved through 60 mesh and observations were made following (Kokoshi*et al.*, 1958).

Method

About 10 g of powdered drug was taken in petridish and treated separately with different reagents viz., methanol, 1N methanolic sodium hydroxide, ethanol (70% v/v), 1N ethanolic sodium hydroxide, 1N HCl, 50% sulphuric acid, 50% nitric acid and 5% potassium hydroxide, acetic acid, etc. These were observed under short UV (254 nm), long UV (365 nm) and visible light.

Table 3.Fluorescence analysis.

Treatments	***Observations***		
	Day light	***Long UV***	***Short UV***
Powder as such	Green	Mahogony	Dark green
Powder + 1N NaOH (aqueous)	Brownish green	Brown	Fluorescent green
Powder + 1N NaOH (Methanolic)	Green	Brown	Dark green
Powder + 1N H_2SO_4	Green	Brownish green	Dark green
Powder + 1N HNO_3	Orange red	Pale brown	Fluorescent green
Powder + 1N HCl	Green	Golden brown	light green
Powder + Acetic acid	Green	Deep orange	Fluorescent green
Powder + Iodine	Brownish green	Golden brown	Fluorescent dark green
Powder + $FeCl_3$	Light green	Brown	Fluorescent dark green
Powder + KOH	Light green	Golden brown	Light green

6.7Determination of extractive values

Extraction values are useful for determination of crude drugs & it gives an idea about the nature of the chemical constituents present. The solvent used for the extraction should be in position to dissolve appropriate quantities of desired substances. (Practical Pharmacognosy text book by Dr.B.Durai Swamy and Prof. K.Jayaveera, S.Chand).

Procedure

About 50 gms of air dried coarse powdered drug was weighed and extracted separately using soxhelt apparatus with solvents like Pet. Ether, Benzene, Chloroform, Methanol and Water. The extract was filtered and the filtrate was evaporated to dryness in a tarred flat bottomed swallowed dish, dried at 105°C& weighed. The %of the extractive values was calculated with reference to the air-dried drug. (Practical Pharmacognosy text book by Dr.B.Durai Swamy and Prof. K.Jayaveera, S.Chand).

Table 4.Extractive values of Roots of *TamilnadiaUlignosa (Retz.) Tirven&Sastre*

Solvent	Extractive value % W/W	Color	Odour	Consistency
a) Pet ether	1.84	Light brown	Characteristic	Greasy
b) Benzene	0.21	Dark brown	Characteristic	Non greasy
c) Ethyl acetate	0.23	Brown	Characteristic	Greasy
d) Chloroform	0.9	Light brown	Characteristic	Non greasy
e) Methanol	8.7	Brown	Characteristic	Sticky greasy
f) Water	5.6	Cream brown	Characteristic	Greasy

LB –Light Brown,
DB –Dark Brown
CB – Cream Brown

PHYTOCHEMICAL ANALYSIS OF THE ROOT OFTAMILNADIA ULIGNOSA (Retz.) Tirven&Sastre (RUBIACEAE)

7.1 Introduction

Phytochemical constituents have played a major role as basic source for the establishment of several pharmaceutical industries. Many medicinal plants occurring in India are yet to be subjected to various chemical investigations, which may help in the discovery of several new drugs. To investigate such chemical constituents from plants, phytochemical screening is required. Broadly, chemical constituents in plants may be divided into major groups viz., primary and secondary chemical constituents. Primary constituents are the basic metabolites of plants such as carbohydrates, proteins, lipids, cellulose and chlorophyll which are distributed in almost all the plants. Secondary chemical constituents are selective and vary considerably from plant to plant and even within the species or varieties of same genus. Secondary chemical constituents are chiefly responsible for the biological activities of plants or drugs.

7.2 Materials and Methods

Petroleum ether, chloroform, methanol, distilled water, hydrochloric acid, sulphuric acid, acetic anhydride, sodiumnitroprusside, pyridine, potassium hydroxide, phenolphthalein, ferric chloride, gelatin, sodium chloride, lead acetate, bromine, magnesium, benzene, silica gel, Mayer's reagent Dragendroff's reagent, Wagner's reagent, Hager's reagent, alcoholic α-Naphthol, Fehling's reagent, Benedict's reagent, Millon's reagent, Biuret reagent, Ninhydrin solution.

7.3 Extraction

The sun-dried and ground aerial parts of TamilnadiaUlignosa (Retz.) Tirven&Sastre (600g) were extracted with methanol (MeoH 4L)using Soxhletion for five days. The extract was concentratedby evaporation underreducedpressureat 40^0C. The Methanolic extract extract of TamilnadiaUlignosa (Retz.) Tirven&Sastre. Was made to 90% aq. Methanolic extract extract and subjected to solvent partitioning with petroleum ether (PE).The resulting aqueous Methanolic extract extract was further partitioned with chloroform(CHCl3) and finally with ethyl acetate (EtOAc). All solvent extracts were concentrated by evaporation under reduced pressure at 40 ^{0}C.

Fig No. 4. Extraction of *TamilnadiaUlignosa (Retz.) Tirven&Sastrein* Soxhlet apparatus

7.4 Phytochemical Analysis

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of the various constituents as per the standard procedures.(Kokate and Trease and Evans)

(1) Detection of Alkaloids

Small portions of solvent-free chloroform, alcohol and aqueous extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents.

a. **Mayer's test**: Filtrates were treated with potassium mercuric iodide (Mayer's reagent) and the formation of cream coloured precipitate was indicates the presence of alkaloids.

b. **Dragendroff's test**: Filtrates were treated with potassium bismuth iodide (Dragendroff's reagent) and formation of reddish brown precipitate was indicates the presence of alkaloids.

c. **Wagner's test**: Filtrates were treated with solution of iodine in potassium iodide (Wagner's reagent) and formation of brown precipitate was indicates the presence of alkaloids.

d. **Hager's test:** Filtrates were treated with a saturated solution of picric acid (Hager's reagent) and formation of yellow precipitate was indicates the presence of alkaloids.

(2) Detection of Carbohydrates and Glycosides

Small quantity of alcohol and aqueous extracts were dissolved separately in distilled water and filtered. The filtrate was subjected to various tests to detect the presence of different carbohydrates.

a. **Molisch's test:** Filtrates were treated with alcoholic solution of α -Naphthol and a few drops of conc. Sulphuric acid were added through the sides of the test tube. The formation of violet ring at the junction of the liquids was indicates the presence of carbohydrates.

b. **Fehling's test:** Filtrates were treated with few ml of dilute hydrochloric acid and heated on a water bath for 30 minutes. After hydrolysis the solutions were neutralized with sodium hydroxide solution. To the neutralized solutions, equal quantities of Fehling's A & Fehling's B solutions were added and heated on a water bath for a few minutes. Formation of red-orange precipitate was indicates the presence of reducing sugars.

(3) Detection of Phytosterols

Petroleum ether, chloroform, methanol and aqueous extracts were refluxed separately with solution of alcoholic potassium hydroxide till complete saponification took place. Saponified mixtures were diluted with distilled water and extracted with solvent ether. Ethereal extract was evaporated to dryness and the residue subjected to Liebermann-Burchard's test.

Liebermann-Burchard's test

Ethereal residues were treated with a few drops of acetic anhyride; boiled, cooled, and 1 ml of sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two liquids and green colour in the upper layer indicates the presence of steroids and triterpenoids.

(4) Detection of Fixed oils and Fats

a. **Spot test:** A small quantity of petroleum ether and benzene extracts were pressed separately between two filter papers. Formation of oil stains on the filter paper was indicates the presence of fixed oil.

b. **Saponification test:** A few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of petroleum ether or benzene extract along with a drop of phenolphthalein. Mixture was heated on a water bath for 1 to 2 hrs. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

(5) Detection of Saponins

Foam test: About 1 ml of alcohol and aqueous extracts were diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of any froth above the surface was indicates the presence of saponins.

(6) Detection of Phenolic compounds and Tannins

Small quantities of alcohol and aqueous extracts were diluted separately in water and were tested for the presence of phenolic compounds and tannins.

a. **Ferric chloride test:** To the test solutions, a few drops of 5% ferric chloride solution were added. Formation of a bluish-black or greenish-black colour was indicates the presence of phenolic compounds and tannins.

b. **Gelatin test:** To the test solutions a few drops of 1% gelatin solution in 10% sodium chloride were added. Formation of white precipitate was indicates the presence of tannins.

(7) Detection of Proteins and Free Amino Acids

Small quantities of alcohol and aqueous extracts were diluted separately in waterand tested for the presence of proteins and free amino acids by subjecting the extracts to various tests.

a. **Millon's test:** To 2 ml of the test solutions, 2 ml of Millon's reagent were added and heated. Formation of white precipitate that gradually turns red was indicates the presence of proteins and amino acids.

(8) Detection of Gums & Mucilage

About 10 ml of aqueous extract was added to 25 ml of absolute ethanol with constant stirring. Precipitate was examined for its swelling properties and for the presence of carbohydrates.

(9) Detection of Flavonoids

a. Shinoda Test: To the test solutions, a few fragments of magnesium metal were added along with concentrated hydrochloric acid, and heated. Formation of magenta colour was indicates the presence of flavonoids.

Table 5. Preliminary Phytochemical analysis of various extracts of Tamilnadia Ulignosa (Retz.) Tirven & Sastre(Rubiaceae).

SL.No.	Test	Methonal	Water
1.	Carbohydrates	+	+
2.	Alkaloids	-	-
3.	Glycosides	+	+
4	Phenols & Tannis	+	+
5	Acidic Compounds	-	-
6.	Flavanoids	+	+
7.	Saponins	+	+
8.	Proteins andAminoacids	-	-
9.	Gums and Mucilages	+	+

"+" Represnets **Presence"-"** Represents **Absence**

The phytochemical analysis helps in formulating pharma copoeal standards.The chief phytochemical presentin the different extracts, of Tamilnadia Ulignosa (Retz.) Tirven & Sastre were flavonoids, polyphenols, alakolids, steriods, tannis,Carbohydrates,saponins andaminoacids. Methonal and water were used for extraction for further studiesbecause percentage yield of both solvents seemed to be more compared to other solvents.

8.TOXICOLOGICAL STUDIES OF METHANOL EXTRACT ROOTS OF TAMILNADIA ULIGNOSSA (Retz.) Tirven&Sastre (RUBIACEAE)

A thorough literature survey indicates that Tanins, Saponins Glycosides, Phenols, Gums and Mucilagesflavanoids are responsible for the therapeutic efficacy of plant as an Diuretic agent. Hence Methanol extract of Ranida Ulignosa (Retz.) Tirven & Sastre (Rubiaceae) which contains rich quantities of Tanins, and flavanoid compounds was ued further studies of toxicological and pharmacological studies.(R.Naganjaneyulu et al., 2011)

8.1 Acute oral toxicity studies of Methanol extract of Tamilnadia Ulignosa (Retz.) Tirven & Sastre.

The acute toxic class method is a step wise procedure with six animals of a Male rats per step. Depending on the mortality or moribund status of the animals and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of animals while allowing for acceptable data based scientific conclusion. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). The starting dose level of EEGS was 2000 mg/kg body weight p.o as most of the crude extracts posses LD 50 value more than 200 mg/kg p.o. Dose volume was administered 0.2ml per 100gm body weight to overnight fasted rats with were *ad libidum*. Food was withheld for a further 3-4 hours after administration of METU and observed for signs for toxicity. The body weight of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted. Hence, 1/20th (100mg/kg), 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study. (R.Naganjaneyulu et al., 2011)

Acute Toxicity

Acute toxicity is usuallydefined as the adverse changes occuring immediately or ashort time following a single or short period ofexposure to a substance or as adverse effects occuring within a shorttime of administration of a single dose ormultiple doses given within 24 hr.(R.Naganjaneyulu et al., 2011)

- **Relationship between Dose and Toxicity**

Toxicity isthe study of the relationshipbetween dose and its effects on the exposed organism. The Chief Criterion regarding the toxicity of a chemical is the dose, i.e. the amount of exposure to the substance.Paracelsus,who lived in the 16th century, wasthe first person to explain dose-response relationship of toxic substances.(R.Naganjaneyulu et al., 2011)

Dose-Response Model

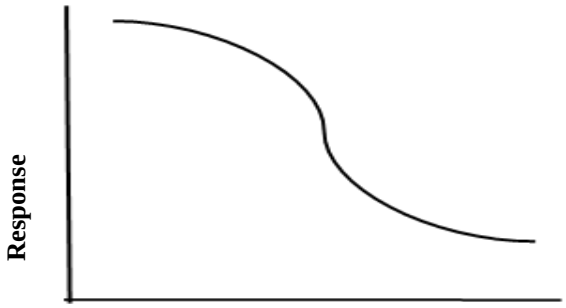


Fig No.5. Dose- Response Curve

Factors Influencing Toxicity

Toxicity of a substance can be affected by different Factors:

Pathway of administration (Whether the toxin is applied to the skin, ingested, inhaled, injected).

- The time of exposure (a brief encounter or long term).
- The number of exposures (a single dose or multiple doses over time).
- The physical overall health and many others.
- Acute exposure: Single exposures to a toxic substance which may result in severe biological harm or death, acute exposure are usually characterized as lasting no longer than a day.
- Chronic exposure: Continues exposure to a toxin over an extended period of time, often measured in months or years can cause irreversible side effects.

8.2 Materials and methods

Single dose is administered through the oral route by conducting acute toxicity. The administration of drug upto 14 days and study the behavioral changes.

Number of animals required: 6 Rats (male)

Number of Groups: 4 Groups (6 animals each Group)

Dose Levels: 2000mg/kg body weight of the animals.

Study Duration: 14days

Extract Sample: Methanol extract of TamilnadiaUlignosa (Retz.) Tirven&Sastre

Animals: Wistar albino rats (150-200g),obtained from the Nirmala Collegeof Pharmacy house and they were housed under standard laboratory conditions and were fed commercial rat feed (Lipton India., Mumbai, India) and boiled water, ad libitum. All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee (IAEC) of CPESEA NICP. (R.Naganjaneyulu et al., 2011)

8.3 Methodology

Acute oral Toxicity Study: the procedure was followed by using OECD 423 (Acute Toxic Class Method). The Acute Toxic class method is a step wise procedure with three rats of a single sex per step. Dependingon the mortality or morbidity status of the rats and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of rats while allowing for acceptable data based scientific conclusion.(R.Naganjaneyulu et al., 2011)

- **Experimental Procedure:**

The Acute toxicity studies for methanol extract of TamilnadiaUlignosa (Retz.) Tirven&Sastre Roots were performed using albino rats.The animals were fasted overnight prior to the experiment and maintained under standard conditions. The crudedrug methanolic extract administrated orally in increasing dose and found safe up to dose of 2000mg/kg for all extracts.

- **Preparation of Dose:**

Methanolic extract of Roots of TamilnadiaUlignosa (Retz.) Tirven&Sastre was suspended in distilled water, to prepare a dose of 2000mg/kg body weight of animal and administered1ml/100gm body weight of the animal.

HealthyAlbinowistar rats weighting 150-200g maintained under standard laboratory conditions were used for acute toxicity test according to organization for Economic Cooperation and Development (OECD) guideline 423(OECD Guideline,2000). A total of six rats, two groups were used which received a single oral-dose of 2000mg/kg BW of crude methanolic extract of TamilnadiaUlignosa (Retz.) Tirven&Sastre. The rats were kept overnight fasting prior to administration of crude extract by oral gavage. Food was withheld for further 3-4h. The rats were observed individually at least once during the first 30 min after dosing, then periodically during first 24hr (with special attention during the first 4hr) and daily thereafter for a period of 14 days. Daily observations on the changes in skin and fur, eyes and mucus membrane(nasal),respiratory rate, 189 circulatory signs (heart rate and blood pressure),autonomic effects (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes were noted (OECD Guideline, 2000). The results were illustrated in table 6.(R.Naganjaneyulu et al., 2011)

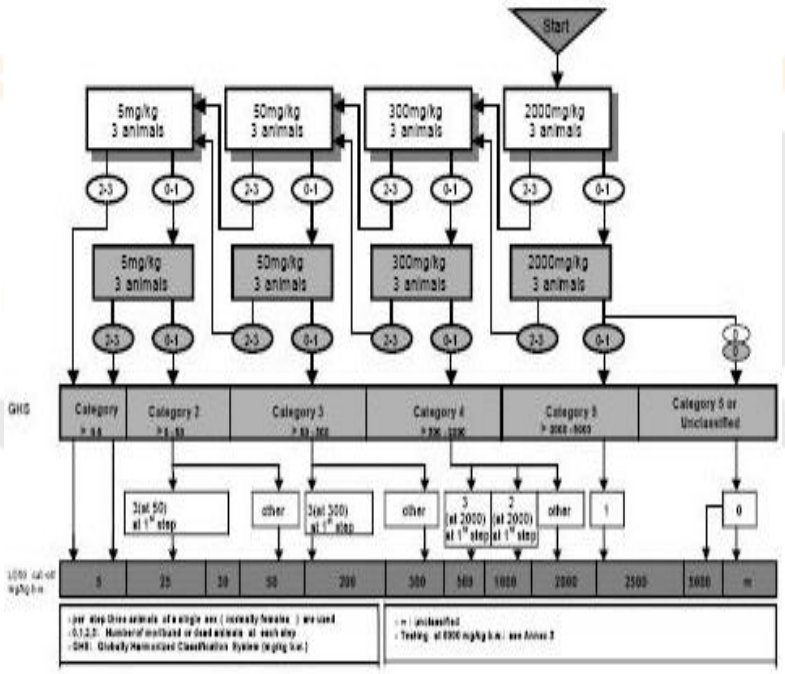


Fig No.6. Flow Chart of acute toxic class method (OECD Guideling 423) Starting dose of 2000mg/kg Body weight.

Animals were observed continuously for 2 hrs under the following aspects.

Behavioral Profile: Alertness, Restlessness, Irritability and fearfulness.

Neurological Profile: Spontaneous activity, touch, response and gait

Autonomical Profile: Defecation and urinate

The mortality and morbidity was observed after 24 hrs

Table6.Acute Toxicity test Studies of METU

Sl.No.	Groups	Dose/Kg BW	Weight of Animals		Signs of Toxicity	Onset of Toxicity	Duration of Study
			Before Test	After Test			
1	METU	2000 mg	167 g	168 g	No signs of Toxicity	Nil	14days
2	METU	2000 mg	187 g	185 g	No signs of Toxicity	Nil	14days
3	METU	2000 mg	172g	174 g	No signs of Toxicity	Nil	14days
4	METU	2000 mg	160 g	166 g	No signs of Toxicity	Nil	14days
5	METU	2000 mg	200 g	203 g	No signs of Toxicity	Nil	14days
6	METU	2000 mg	212 g	210 g	No signs of Toxicity	Nil	14days

8.4 Results and Discussion

The methanolic extract of TamilnadiaUlignosa (Retz.) Tirven&Sastre(METU) was studied for acute toxicity at a dose of 2000mg/kg in albino rats. The extract was found devoid of mortality of the animals. The acute toxicity studies were performed and the results showed no sign of toxicity till 14 days at a starting dose of 2000mg/kg body weight. Hence, it has been that the MERU is to be safe even at the dose of 400mg/kg, p.o.

The body weight of the rats before and after administration was noted and the changes in the body weight were not so prominent. No Changes in skin and fur, eyes, mucous membrane, respiratory, circulatory, autonomic and central nervous system, motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation diarrhea, lethargy, sleep and coma were noted. They onset of toxicity and signs of toxicity also not there. In this study there was no toxicity death were observed at the dose of 2000 mg/kg b.w. The acute toxicity study in rats showed that at 2000 mg/kg dose, the plant is safe for consumption and for medicinal uses (Table 6).(R.Naganjaneyulu et al., 2011).

9. DIURETIC ACTIVITY OF ROOTS OF TAMILNADIA ULIGNOSA (Retz.) Tirven&Sastre (RUBIACEAE)

9.1 Introduction

Diuretic Definition

These are Drugs which cause a net loss of Na^+ and water in urine.

Diuretics are among the most widely prescribed drugs. Application of diuretics to the management of hypertension has outstripped their use in edema. Availability of diuretics has also had a major impact on the understanding of renal physiology.

Classification of Diuretics

There are 3 types of diuretic medicines. Each type works a little differently, but they all lower the amount of salt and water in your body, which helps to lower your blood pressure (K.D.Tripati Text book Pharmacology 5th Volume).

1. Potassium-sparing diuretics (Weak efficacy diuretics)

Potassium-sparing diuretics are used to reduce the amount of water in the body. Unlike the other diuretic medicines, these medicines do not cause your body to lose potassium.

Ex : Carbonic anhydrous inhibitors :Acetazolamide

Aldosterone antagonist: Spiranolactone, Amiloride etc.

Osmotic Diuretics: Mannitol, Isosorbide Glycerol.

Xanthins : Theoplhylline

Among these commonly used brand names in the United States: Aldactone (spironolactone), Dyrenium (triamterene), Midamor (amiloride)

2. Thiazide diuretics (Medium efficacy diuretics)

Thiazide diuretics are used to treat high blood pressure by reducing the amount of sodium and water in the body. Thiazides are the only type of diuretic that dilates (widens) the blood vessels, which also helps to lower blood pressure.

Ex: Chlorothiazide, Hydrochlorothiazide, Benzthiazide etc.

Among these commonly used brand names in the United States: Aquatensen (methyclothiazide), Diucardin (hydroflumethiazide), Diulo (metolazone).

3. Loop-acting diuretics (High Ceiling diuretics)

Loop-acting diuretics acts on the kidneys to increase the flow of urine. This helps reduce the amount of water in your body and lower your blood pressure.

Commonly used brand names in the United States: Bumex (bumetanide), Demadex (torsemide), Edecrin (ethacrynic acid), Lasix (furosemide), Myrosemide(furosemide)

Signs and symptoms

Electrolyte disturbances are the common adverse effects seen with loop diuretics. They are.

1. Hypokalaemia (↓ K^+ levels)
2. Hyponatraemia
3. Hypokalaemic alkalosis
4. Hypocalcaemia
5. Ottoxicity
6. GIT: Nausea, vomiting diarrhoea

Mechanism of action

Loop Diuretics bind to luminal side of Na^+ - K^+ - $2Cl^-$ co transporter and block its function. There is an increased excretion of Na^+ and Cl^- in the urine. Furosemide also has weak carbonic anhydrase-inhibiting activity, hence, increases the excretion of HCO_3^- and PO_4^-. The tubular fluid reaching the DCT contains large amount of $_{Na}^+$. Hence, more Na^+ exchanges with K^+ leading to K^+ loss. They also increase the excretion of Ca^{2+} and Mg^{2+}.A Loop diuretic are administered by oral, i.m. and i.v. routes, and rapidly absorbed through the gastrointestinal tract. The duration of action of furosemide is 2-3 hours (K.D.Tripati Text book Pharmacology 5th Volume).

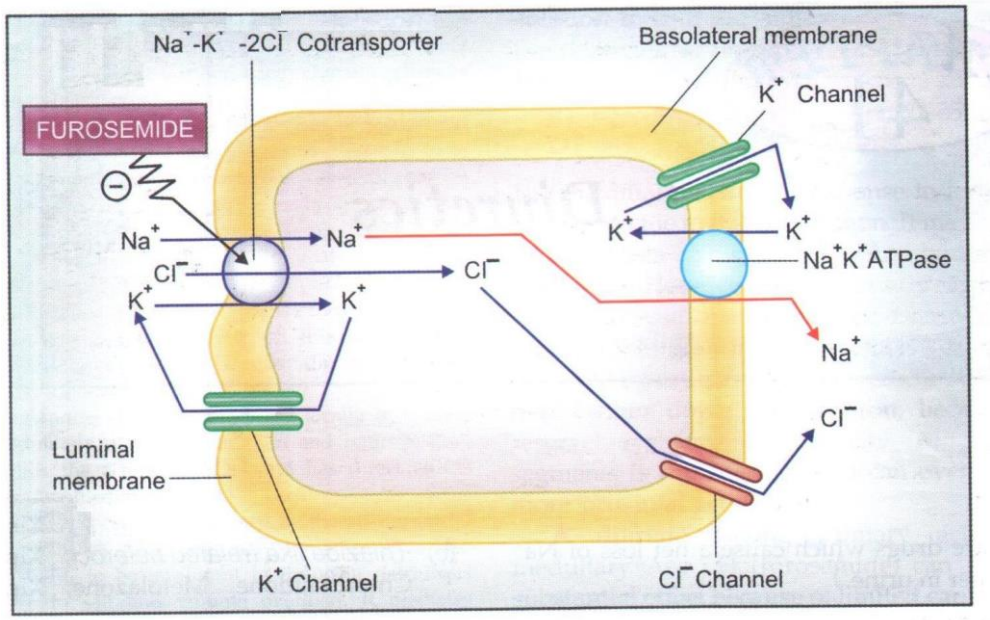


Fig No.7. Mechanism of Salt Reabsorption in the thick ascending limb of loop of Henle (AscLH) cell, and site of action of furosemide on the Na^+ -K^+-$2Cl^-$ cotransporter.

Uses :

Edema : Acute Pulmonary edema, cerebral edema.

Hyper tension

Hyper calcaemia

Prevents the formation of stones in kidneys.

Forced diuresis in the poisoning

Herbal drugs as diuretics

A diuretic is a substance that helps the body retains water. It prevents the kidneys and bladder from removing and eliminating water from the body too quickly. Diuretic medicines are used to treat bed-wetting, incontinence and similar conditions. Some herbs have natural diuretic properties. When adding any herbal supplement to your diet, however, remember to first check with your doctor to ensure the supplement will not interfere with your other medications or conditions and that the symptoms for which you are taking the supplement do not require further medical intervention.

Table7.List of plant used as the Diuretics

Sl.No.	Botanical Name	Family	Part Used	Uses
1.	*Aervalanata*	Rutaceae	Whole plant	diuretics
2.	Crataevanurvula	Capparidaceae	Fruit	diuretics
3.	Orthosiphon stamineus	Laminaceae	Whole plant	diuretics
4.	Strychnospotatorum	Loganiaceae	Seed	diuretics
5.	Aervajavanica	Amaranthaceae	Fresh roots	diuretics
6.	Carica papaya	Caricaceae	Seed	diuretics

7.	Ficus religiosa L	Moraceae	Latex	diuretics
8.	Pedalium murex Linn	Pedaliaceae	Dried fruits	Diuretics

9.2 Materials and methods

- **ExtractSample:** Methanolic extract of TamilnadiaUlignosa (Retz.) Tirven&Sastre
- **Standard Drug:** Furosemide
- **Animal:** Male Wistar albino rats

Wistar albino rats (150-200g), obtained from the Nirmala College of Pharmacy house and they were housed under standard laboratory conditions and were fed commercial rat feed (Lipton India., Mumbai, India) and boiled water, ad libitum. All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee (IAEC) of CPESEA NICP. Saurabh Srivastav et al 2011,1(2) 97-102.

Experimental Design

Group I: Control group received normal saline (25 ml/kg p.o.)

Group II: Standard group received furosemide (100 mg/kg wt., p.o.)

Group III: Received 200mg/kg METU suspended in 0.9% saline solution.

Group IV: Received 400mg/kg METU suspended in 0.9% saline solution

Test for diuretic effect:

Lipschitz test as described by Lipschitz et al., (1943) was followed. Albino rats weighing 130–160 gm were used. In all the groups, rats of approximately equal weight were allocated. Six hour prior to the experiment, food was withdrawn with water *ad libitum* till the start of the experiment. Since numbers of metabolic cages were limited, only one rat per group was used per day. The drugs were dissolved in 0.9% NaCl (normal saline). It has been stated by Haravey (1966) that the indigenous drugs when tested on water-loaded animals, there was no appreciable difference between control and test groups; perhaps due to the fact that the water itself acts as a mild diuretic. It has also been mentioned by Dollery (1999) that the administration of normal saline maintains the extracellular fluid volume and corrects any pre-existing losses. The drug solutions were given by gavage at a volume of 25 ml/kg at approximately body temperature. The drugs given for the various groups were as follows:

Control group- normal saline (25 ml/kg),

Standard group- furosemide (100 mg/kg).

Test 1- Methanolic extract of Tamilnadiaulignosa(200 mg/kg),

Test 2- Methanolic extract of Tamilnadiaulignosa(400 mg/kg) .

Soon after feeding, the animals were placed separately in metabolic cages provided with a mesh wire bottom and a funnel to collect the urine. No water or food was given during the time of the experiment. Both in control and test drug treated groups, after 5 h is not sufficient for collection of urine as the volume excreted was minimal or not at all. Thus, overnight (18 h) urine was collected for all the groups tested even though the urine output in furosemide treated group was enough. At the end of the experiment, residual urine from the bladder was expelled by gentle pressure on the abdomen and simultaneously pulling the base of the tail. The volume was measured and urine sample kept in refrigerator until Na+, K^+ and Cl^- levels were estimated. The urine samples were kept without adding any preservatives (Young and Bernes, 2001).

Quantitative estimation of Na^+,K^+ and Cl^-:

The electrolytes (Na^+,K^+ and Cl^-) were determined by colorimetry. Quantitative estimation of Na^+,K^+ and Cl^- were done by following the instructions as directed in the kit used (Medsource Ozone Biomedicals Pvt Ltd). The kit was meant for human serum electrolyte estimation. The electrolyte concentration in urine was higher than in serum (Varley, 2005) and it was even higher in rats than in

humans as observed by previous researchers (Durairaj, et al., 2007; Johnson, 2007). For determination of rat urine, trial and error method was followed in dilution of urine. Chloride kit was stored at room temperature and sodium-potassium kit was stored at 2–8°C. All glassware's and cuvette were washed with deionised water and dried in hot air oven before use.

Sodium assay

Step 1- Precipitation of Na^+ and protein:

1 ml of precipitating reagent (for sodium) was pipetted into clean, dry centrifuge tubes: one for the standard reagent labeled as S and the other 4 test tubes labeled as T, one each for all the experimental groups namely, T1 (Control), T2 (Standard drug-furosemide)T3 (Test-200 mg/kg), T4 (Test-400 mg/kg) . Then 10 µl of standard reagent was added into test tube S and 10 µl of urine to all the T series test tubes. The solution was shook vigorously and incubated at room temperature for 5 min, then centrifuged at 2000–3000 r.p.m. for 2 min to obtain a clear supernatant.

Step 2- Colour development:

1 ml of colour reagent (for sodium) was pipette into each of the clean, dry test tubes labeled B for Blank, S for Standard reagent and 4 test tubes labeled T1 to T4 as above. 20 µl of the appropriate supernatant from step1 was then added into the corresponding test tube S and tubes of the series T. Into test tube B, 20 µl of the precipitating reagent (for sodium) was added. Pipetting of sodium precipitating reagent (in step 1) and quick transfer of supernatant (in step 2) was followed. The solution was mixed well and allowed to stand at room temperature for 5 min, then the absorbance of Blank (Abs B), Standard (Abs S) and Test series (Abs T1, Abs T2, Abs T3, Abs T4.) against distilled water on a photo colorimeter with 530 nm within 10 min was measured.

Potassium assay:

1 ml of boron reagent for potassium was pipetted into dry clean centrifuge tubes labeled Standard (S) and Test (T)- T1, T2, T3, T4 as described for step 1 in Na^+ assay. 50 µl of standard reagent was added into the test tube S and the same amount of corresponding urine sample was added to the T series of test tubes. The solution was mixed well; incubated at room temperature for 5 min, then the absorbance of Standard (Abs S) and that of Test series (Abs T1, Abs T2……Abs4) against distilled water on a photo-colorimeter with 620 nm within 10min was measured. The urine Na^+ and K^+ for each group/test series was then measured by the following formula:

Na^+ in mMol/L = [(Abs of B - Abs of T) / (Abs of B - Abs of S)] × 150,

K^+ in mMol/L = (Abs of T / Abs of S) × 5.

This procedure is linear up to 200 m Mol/L for Na^+ and up to 7 m Mol/L for K^+. If values exceed these limits, urine samples were diluted with deionised water and assay repeated, and the final value was calculated using appropriate dilution factor.

Quantitative estimation of Cl^-:

Urine specimen was diluted 1+1 with distilled water. 1 ml of colour reagent was pipette into test tubes labeled Blank (B), Standard (S) and Test (T) - T1, T2, T3, and T4. 10 µl of chloride standard was added to the test tube S while 10 µl of the appropriate urine sample was added to the corresponding T series test tubes. The solution was mixed well and the absorbance of standard (Abs S) and Test (Abs T) against Blank (Abs B) were read at 530 nm. The Cl^- content was calculated by using the given formula:

Cl^- in mMol/L = (Abs of T / Abs of S) × 100.

Linearity for this procedure is 140–150 mMol/L. The final value was calculated by multiplying the dilution factor 2.

Statistical Analysis

The data statistically analysed using one way ANOVA followed by Tukeykramers multiple range test for individual comparison of group's control. 'p' values below 0.05wereconsidered as significant. Here all values of analysis are expressed as mean ±SEM.

9.4 Results and discussions

Preliminary phytochemical screening of methanolic extract of TamilnadiaUlignosa (Retz.) Tirven&Sastre revealed the presence of Tannis,Flavanoids carbohydrates glycosides phenols saponins gums and mucilages. The results of diuretic parameters for preliminary phytochemical screening of extracts are shown in table 8, so far as an impact study on diuretic induce by furosemide is concerned, the drug also exhibits a significant effect to increase excretion of Na^+k^+ ions at doses of 200mg/kg and 400mg/kg and this

maximum Na^+k^+ ions excretion at 400mg/kg. The animals show significant increase of Na^+k^+ ions after 24hrs of administration of furosemide. Administration of the drug on 12th of furosemide administration to increase the excretion of Na^+K^+ ions concentration in the body. But one hour after administration of the plant extracts (TamilnadiaUlignosa (Retz.) Tirven&Sastre) as well as the standard (Furosemide) increases the excretion of $Na^+ K^+$ ions so the Tamilnadiaulignosa possess the diuretic activity.

Diuretic activity

Effect on urine volume

Results are shown in the table :8 The methanolic extract of the whole plant of Tamilnadiaulignosa at a dose of 400 mg/kg BW show marked diuresis during the 18 hr of the test (Tamilnadia ulignosa8.133±0.3575 ml versus control 3.367±0.029ml, whereas in case of standard the volume was found to be 8.750±1.0423ml; $P < 0.05$).

The methanolic extract of Tamilnadiaulignosa significantly increased urinary output to that of the control (Tamilnadia ulignosa85.42±1.425 ml versus control 3.367±0.29ml; $P < 0.05$), but the effect was much less than that of furosemide (8.750±1.0423ml).

Table 8.Effect of Methanolic extract of TamilnadiaUlignosa on urine excretion and Ionic concentration in Rats.

Treatment	Dose (oral)	Urine Volume (ml/kg)	Urine electrolytes (mMol/L/18hr)			
			Na^+	K^+	Cl^-	Na^+/K^+ Ratio
Normal saline	(25ml/kg) p.o.	3.367±0.029	60.17±1.400	21.33±2.525	181.7±13.58	2.824
Furosemide	(100mg/kg) i.p.	8.750±1.0423****	140.25±9.102****	128.8±10.65****	428.3±22.42****	1.088
METU Test -1	(200 mg/kg) p.o.	5.417±0.6776****	72.00±2.499****	62.50±2.643****	241.2±10.34****	1.152
METU Test -2	(400 mg/kg) p.o.	8.133±0.3575****	85.42±1.425****	107.3±3.861****	273.8±9.799****	0.796

Statistical analysis by ANOVA and Tukey kramers Multiple comparison Test. Results are expressed as mean ± standard error, n = 6 in each group.

****Significantly difference compared to control group at $p < 0.05$. ***Significantly difference compared to control group at $p < 0.0001$.

p.o : per oral

i.p : intra peritoneal

METU : Methanolic Extract of TamilnadiaUlignosa

Fig. No. 8 Total urine volume(ml)

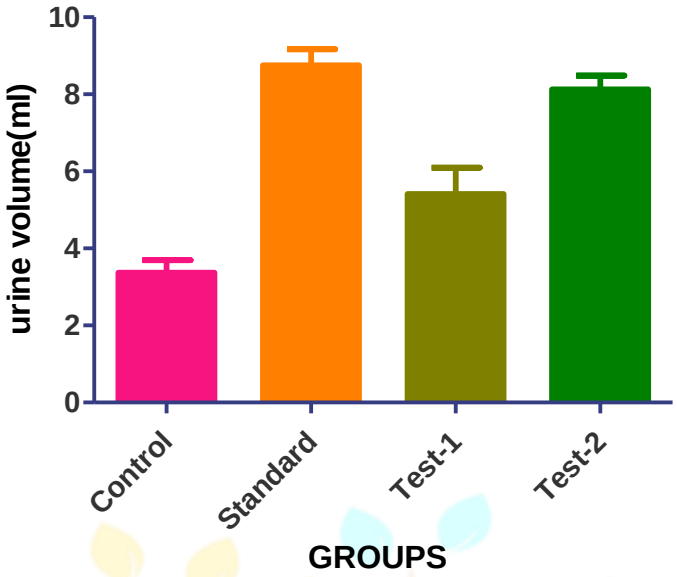


Fig.No.9 Na^+Concentration(mMol/L)

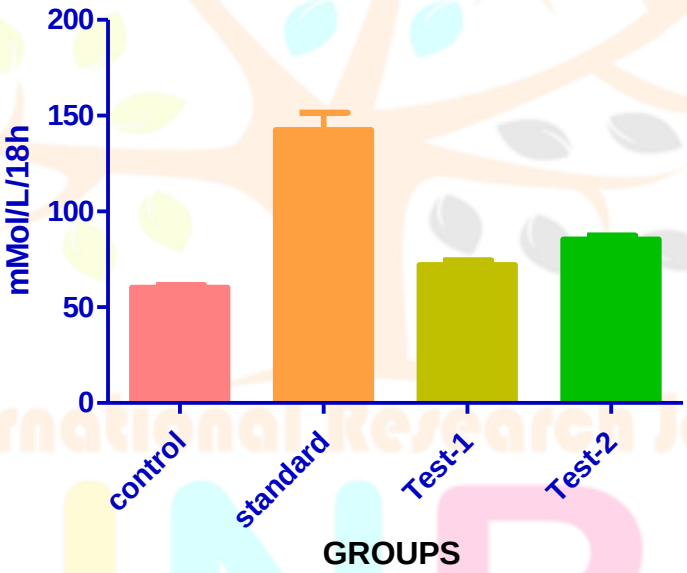


Fig.No.10 K^+Concentration m(Mol/L)

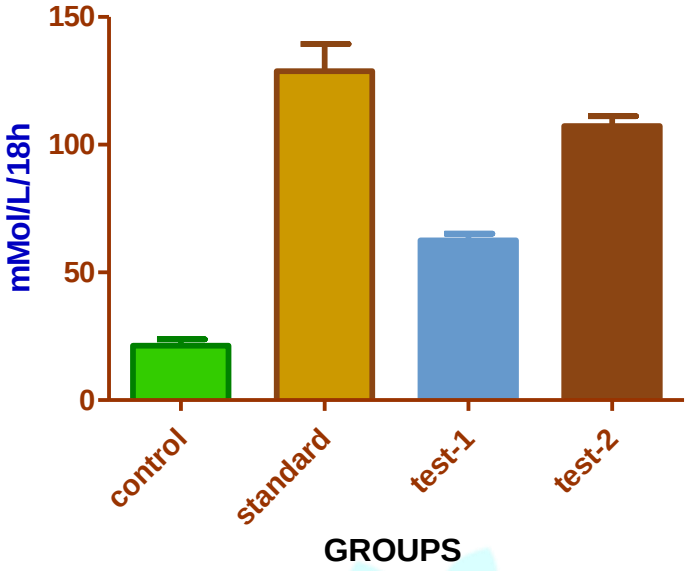
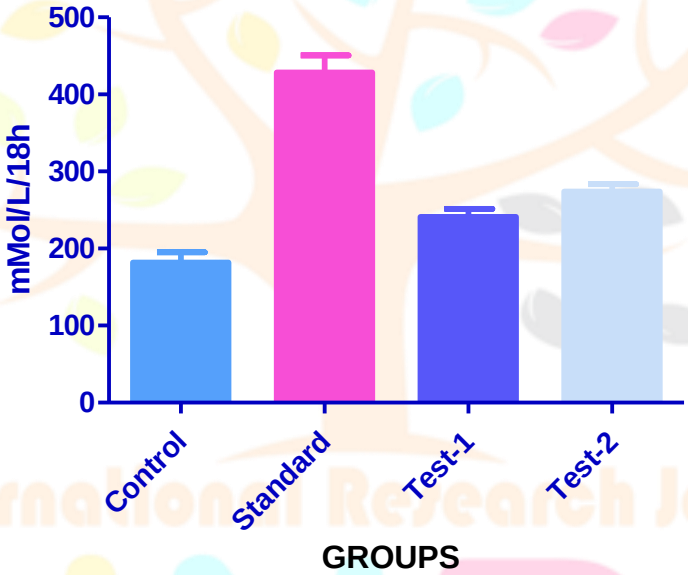


Fig.No.11 Cl^-Concentration (mMol/L)



10. SUMMARY AND CONCLUSION

Plant kingdom is a true source of natural medicines. The human population suffering with more number of diseases. So a considerable number of those potentially useful species may be examined scientifically and clinically before being compressed, burned or powdered into suitable formulations. There are still many new drugs to be discovered, but to locate them has become a race against time since it involves a detailed study of different disciplines of science. Thus a structured study, which will aid in standardization and authentification of the herbal drugs, is of prime importance. Such studies also offer scientific details for solving problems of identification and in laying down standardization parameters for drugs, ultimately resulting in pharmacopoeia for indigenous drugs. The present work is one such: contribution towards achieving this goal.

It is concluded that, scientific parameters based on taxonomical, pharmacognostical and phytochemical studies are essential in identifying and distinguishing the plant from other species. These parameters along with physiochemical constants not only help in standardization of these drugs but also aid in formulating pharmacopoeial standards of drugs. The exomorphic characters have been found to be useful tools to identity the species taxonomically.

The current study evaluated the diuretic potential of TamilnadiaUlignosa roots in Wistar albino rats. The purpose of the present study was to establish the scientific basis for the traditional and the reported folk use of TamilnadiaUlignosa (Retz.) for diuresis. The methanolic extract was given orally in the same way local people practice traditionally. The chemical analysis for the presence of flavonoides, phenolic compounds, triterpenoids, tannins and saponins was also done. The earlier study has reported the presence of these chemicals in the plant. The diuretic assay was done using Lipschitz test which is one of the commonly used standard models. This is probably the first study of the methanolic extract of roots of TamilnadiaUlignosa (Retz.). On the diuretic property. The study indicated that the diuretic activity of the plant extract was apparent in a narrow dose range. The effect was not seen at 200 mg/kg. The dose of 400 mg/kg showed significant increase in urine output and excretion of electrolytes. In the present study, Na^+ 85mMOl/L/18h excretion was less than that of Cl^- 273mMOl/L/18h. From these studies reported that the normal renal physiological values in a rat are a urine volume of 8 to 10 ml/18h with Na^+ excretion of 85 mMol/L/24 h and k^+107mMOl/L/18h. The diuretic activity of the plant extract is relatively modest and slow in onset as compared to the reference drug, (furosemide). The loop diuretic furosemide acts by inhibiting three-ion co-transporter system i.e. the Na^+ /K^+/2Cl^-. The plant extract must have acted in the same way like furosemide as it also cause increase excretion of all these 3 electrolytes. The possibilities in the increased diuretic activity seen with test drug may have resulted from the slow absorption of the active principle in the extract or due to biotransformation to its active metabolite or in vivo stimulation of endogenous diuretic compound. The increasing urine output as well as increasing urine electrolytes excretion at higher doses.

The methanolic extract of TamilnadiaUlignosa (Retz.) Tirven&Sastre was screened for diuretic activity. Methanolic extract showed significant Diuretic effect when compared to control and increases the loss of Na^+ K^+ ions concentration when compared to normal. From this study, it is concluded that roots of TamilnadiaUlignosa (Retz.) Tirven&Sastre (Rubiaceae) Possess Diuretic activity.

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