PHARMACOGNOSTICAL, PHYTOCHEMICAL, EVALUVATION OF SEDATIVE AND HYPNOTIC ACTIVITY ON SEED ARIL OF *Myristica malabarica* Lam.,

A dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

CHENNAI-600 032

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

MASTER OF PHARMACY

IN

PHARMACOGNOSY

Submitted by

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MAY 2017

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CERTIFICATE

This is to certify that the dissertation entitled "**PHARMACOGNOSTICAL, PHYTOCHEMICMISTRY, EVALUVATION OF SEDATIVE AND HYPNOTIC ACTIVITY ON SEED ARIL OF *Myristica malabarica* Lam.,**" submitted by **Reg. No: 261520656** in partial fulfilment of the requirements for the award of the degree of **MASTER OF PHARMACY IN PHARMACOGNOSY** by The Tamil Nadu Dr. M.G.R Medical University, Chennai is a bonafide record of work done by him in the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003 during the academic year 2016-2017 under the guidance of **DR. R. RADHA, M.PHARM., Ph.D.,** Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003.

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DEDICATED TO MY BELOVED

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INTRODUCTION

1. INTRODUCTION

In our modern civilization we are living in a stressful and disarray environment where sleep cycles are easily disturbed and leads to sleep disorders. Sleep disorders such as Insomnia, Narcolepsy, Parasomnia, Sleepwalking, Night terror, etc., arises due to inadequate or lack of sleep[1]. Medicines such as Benzodiazepines and barbiturates are commonly prescribed to treat sleep disorders. However these drugs on the long run causes undesirable side effects and various complication such as drug dependence, disorientation, amnesia, impaired psychomotor activity and drug withdrawal symptoms[2]. In India 1 out of 5 patients i.e. that is 20% of the patients suffer from sleep disorder[3]. Traditional medicinal system is based upon the natural resources such as plants, animals and minerals available in their locality.

India, a birthplace for many traditional medicinal systems which are still followed up to till date by majority of the population. This is due to the flourishment of wide variety of flora and fauna. Many medicinally valuable plants are native to Indian sub-continent. Yet much more plants are still are in investigation or lack detailed study.[4] There is much potential in herbal studies. Since allopathic medicines have many undesirable side effects, modern civilization began to turn its head once again to natural method, i.e. traditional medicinal systems. According to WHO more or less 80% of the people relay to their own native traditional medicinal irrespective to their nation or advancement in modern science[5]. There is still a raise in demand to herbal medicines and people are turning back again to herbal remedy due to their no side effect or less cost. India has epic traditional system such as Ayurveda, siddha, unani, tribal, etc,..These traditional system are followed in India long back before the popularization of western allopathic medicinal system. The Ayurveda and siddha traditional system dates back several thousand years BC.

1.1 HERBAL MEDICINE IN INDIA

India stands first among all the Asian countries in having the knowledge on traditional system related to the use of plant species and diversity of higher plant species, which is the reason for the use of herbs in different forms in alternative systems of medicine[6]. India has about 45,000 plant species. This huge number of medicinal plants species possess important role in health system. Among these, several thousand plants have

been claimed to possess medicinal properties. Although herbal medicine are used in various ailments, often these drugs are improperly used and only few of them have got scientifically documented. Hence plant drug need a detailed study in the light of modern medicine in bringing new herbal chemical entities.

Origin[7]

Plants, animals and human beings have intimate biological relationships for their very basic needs like air, food, fire and fuel, since remote past. The primitive men during the course of their struggle in the forest under the circumstances of object poverty disease and hunger. To liberate themselves from these suffering they should have looked towards the nature (i.e.) plants and this inevitably led to trial and error experimentation and discovery of the healing properties of plants by using wild animals. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected the information on herbs and developed well-defined herbal pharmacopeia. In 20th century, the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. About 25% of the prescription drugs dispensed from plant material

Importance

The natural to assume that plants were not only eaten for food but were also used as a source of medicine. Although written and pictorial records of herbalism cover no more than the past 5,000 to 6,000 years, archeologically records clearly show that the knowledge and practise of herbal medicine was highly developed long before the earliest written accounts known to us were made. Plants form the major healing powers in almost all ancient civilization like Egyptian, Chinese, India, Roman and Greek[8].

Foods and herbs as medicine [9]

The majority of medicinal plants found in the world grow wild. As natural habitats worldwide are degraded, overexploited and destroyed, many plants species including medicinal plants face threats to their survival. It is becoming increasingly important to take care of the areas in which they grow and to share knowledge about their usefulness.

Involving local people from areas where the plants grow is crucial. In the India many people rely on plants and plant – products for food, medicine and shelter. We need to balance health first through proper nutrition with the help of herbs serving as special foods for both body and mind.

Scientific approach to the herbal medicine[10]

The technology involving extraction, purification and characterization of pharmaceuticals from natural sources is a significant contribution to the advancement of natural and physical sciences. The rapid development of phytochemistry and pharmacological testing methods in recent years, new plant drugs is finding their way into medicine as a purified phytochemicals, rather than in the form of traditional galenical preparations. Phytopharmaceuticals or synthetic drugs derived from phytochemicals have to be ultimately incorporated in suitable dosage form which involves the knowledge of dispensing and preparative pharmacy, pharmaceutical technology and analysis. Pharmacognosy is a vital link between Ayurvedic and Allopathic systems of medicines. It provides a system wherein the active principles of crude drugs derived from natural origin can could be dispensed, formulated and manufactured in dosage forms acceptable to the Allopathic system of medicine

1.2 DISEASE PROFILE

Fig. 1 Sleep

1.2.1 Sleep[11]:

Sleep is a state of decreased awareness of environmental stimuli that is distinguished from states such as coma or hibernation by its relatively rapid reversibility. Sleeping individuals move little and tend to adopt stereotypic postures. Although sleep is characterized by a relative unconsciousness of the external world and a general lack of memory of the state, unlike people who have been comatose, we generally recognize when we feel sleepy and are aware that we have been asleep at the termination of an episode.

For clinical and research purposes, sleep is generally defined by combining behavioral observation with electrophysiological recording. Humans, like most other mammals, express two types of sleep: rapid eye movement (REM) and non-rapid eye movement (NREM) sleep. These states have distinctive neurophysiological and psychophysiological characteristics.

Phases of Sleep[12]:

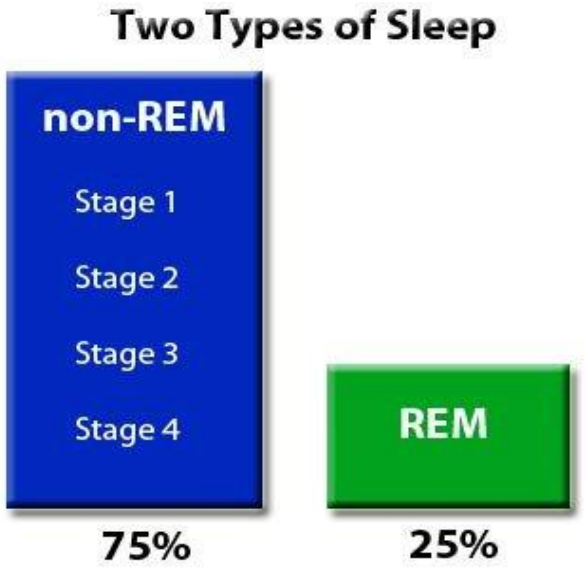


Fig. 2 Types of sleep

- **NREM Sleep:** Also called as orthodox sleep, is characterized by decreased activation of the EEG; in infants it is called quiet sleep because of the relative lack of motor activity. It has four stages.
- **Stage 1 (Dozing):** light sleep where you drift in and out of sleep and can be awakened easily. In this stage, the eyes move slowly and muscle activity slows. During this stage,

many people experience sudden muscle contractions preceded by a sensation of falling.

- **Stage 2 (Unequivocal sleep)**: eye movement stops and brain waves become slower with only an occasional burst of rapid brain waves. When a person enters.
- **Stage 3 (Deep sleep transition):** extremely slow brain waves called delta waves are interspersed with smaller, faster waves.
- **Stage 4 (Cerebral sleep):** the brain produces delta waves almost exclusively. Stages 3 and 4 are referred to as deep sleep or delta sleep, and it is very difficult to wake someone from them. In deep sleep, there is no eye movement or muscle activity. This is when some children experience bedwetting, sleepwalking or night terrors
- **REM sleep:** It derives its name from the frequent bursts of eye movement activity that occur. It is also referred to as paradoxical sleep because the electroencephalogram (EEG) during REM sleep is similar to that of waking. In infants, the equivalent of REM sleep is called active sleep because of prominent phasic muscle twitches.

1.2.2 Sleep Disorder[13-16]:

Fig. 3 Sleep Disorder

The sleep disorders are categorized into primary disorders (i.e. Dyssomnias and Parasomnias), those related to another mental disorder or to a general medical disorder, and those that are substance induced.

Primary Insomnia Disorders:

Primary insomnia is characterized by difficulty in initiating or maintaining sleep or by not feeling rested after an apparently adequate amount of sleep for at least 1 month. It is characterized by excessive daytime worry about being able to fall or stay

asleep. Anxiety tends to perpetuate a vicious cycle of sleeplessness that is aggravated by worry about sleeplessness. Primary insomnia disorders includes dyssomnias and parasomnias. The dyssomnias may occur due to intrinsic sleep disorders, extrinsic sleep disorders and disturbance of the circadian rhythm.

i. Intrinsic Sleep Disorder

A) Psychogenic Insomnisa:

Psychogenic insomnia is characterized by increased mental tension (inability to relax, anxiety, brooding) and excessive concern about sleep itself. Sleep often improves in a new environment (e.g. on vacation).

B) Pseudo Insomnia:

Pseudo insomnia is a subjective feeling of disturbed sleep in the absence of objective evidence (i.e. normal polysomnography).

C) Restless Leg Syndrome:

Restless legs syndrome is characterized by ascending abnormal sensations in the legs when they are at rest (e.g. when the patient watches television, or before falling asleep) accompanied by an urge to move the legs. It is sometimes present as a genetic disorder with autosomal dominant inheritance. Periodic leg movements during sleep are repeated, abrupt twitching movements of the legs that may persist for minutes to hours.

D) Narcolepsy:

Daytime somnolence and frequent, sudden, uncontrollable episodes of sleep (imperative sleep), which tend to occur in restful situations (e.g. reading, hearing a lecture, watching television, long automobile ride. It may be associated with cataplexy (sudden, episodic loss of muscle tone without unconsciousness), sleep paralysis (inability to move or speak when awaking from sleep) and hypnagogic hallucinations (visual or acoustic hallucinations while falling asleep).

E) Obstructive Sleep Apnea:

Obstructive sleep apnea is characterized by daytime somnolence with frequent nocturnal respiratory pauses and loud snoring. Impaired concentration, decreased performance and headaches are also common.

ii. Extrinsic Sleep Disorder:

Sleep may be disturbed by external factors such as noise, light, mental stress and medication use.

iii. Disturbance of Circadian Rhythm:

Sleep may be disturbed by shift work at night or by intercontinental travel (jet lag)

iv. Parasomnias:

These disorders include confusion on awakening (sleep drunkenness), sleepwalking (somnambulism), nightmares, Sleep myoclonus, bedwetting (enuresis), and nocturnal grinding of the teeth (bruxism).

Secondary Sleep Disorders:

i. Psychogenic Sleep Disorder:

Depression (of various types) can impair sleep, though paradoxically sleep deprivations can ameliorate depression. Depressed persons typically complain of early morning awakening, nocturnal restlessness, and difficulty in starting the day.

ii. Neurogenic Sleep Disorder:

Sleep can be impaired by dementia, Parkinson disease, dystonia, respiratory disturbances secondary to neuromuscular disease (muscular dystrophy, acute coronary syndrome), epilepsy (nocturnal attacks), and headache syndromes (cluster headaches, migraine). Fatal familial insomnia is a genetic disorder of autosomal dominant inheritance.

iii. **Sleep Disorder Due to Systemic Disease:**

Sleep can be impaired by pulmonary diseases (asthma, COPD, angina pectoris, nocturia, fibromyalgia and chronic fatigue syndrome).

1.2.3 Symptoms of Sleep Disorder[17]:

i. Depression
ii. Irritability or anxiety
iii. Feeling as if sleep was unrefreshing (non-restorative).

iv. Experiencing excessive daytime sleepiness.
v. General lack of energy.
vi. Difficulty concentrating.
vii. Mood and behavior disturbances such as irritability, aggression, and impulsive behaviors.
viii. Difficulty concentrating.
ix. Forgetfulness.
x. Decreased performance at work or school.
xi. Troubles in personal and professional relationships.
xii. Having accidents at work or while driving fatigued.
xiii. Decreased quality of life.
xiv. Depression.

1.2.4 Causes for Sleep Disorder[18]:

- Psychological problems: depression, anxiety, stress.
- Lifestyle habit such as smoking, caffeine, alcohol, napping and irregular sleeping patterns.
- Medical conditions such as congestive heart failure or prostate enlargement.
- Painful conditions, such as arthritis, shingles.
- Genetics-Researchers have found a genetic basis for narcolepsy, a neurological disorder of sleep regulation that affects the control of sleep and wakefulness.
- Aging-About half of all adults over the age of 65 have some sort of sleep disorder. It is not clear if it is a normal part of aging.
- Medication side effects.
- Chronic use of sedatives, which can cause light, broken sleep patterns.
- Neurological disorders such as Alzheimer's disease, dementia, delirium, Parkinson's disease.
- Not enough exercise.
- Obesity; metabolic syndrome and diabetes.
- Environment problems: Night noise and lights.
- Night-time trips to the bathroom.

1.2.5 Pathophysiology of Sleep Disorder[19]:

The physiological mechanisms regulating the sleep-wake rhythm are not completely known. There is evidence that histaminergic, cholinergic, glutamatergic, and adrenergic neurons are more active during waking than during the NREM sleep stage. Via their ascending thalamopetal projections, these neurons excite thalamocortical pathways and inhibit GABA-ergic neurons. During sleep, input from the brainstem decreases, giving rise to diminished thalamocortical activity and disinhibition of the GABA neurons. The shift in balance between excitatory and inhibitory neuron groups underlies a circadian change in sleep propensity, causing it to remain low in the morning, to increase towards early afternoon (middle siesta), then to decline again, and finally to reach its peak before midnight.

As the margin between excitatory and inhibitory activity decreases with age, there is an increasing tendency towards shortened daytime sleep periods and more frequent interruption of nocturnal sleep. Imbalance between the excitatory and inhibitory neurotransmission with more shift toward excitatory system underlies many of the sleeping disorders.

1.2.6 Management of sleep disorders[20-25]:

- Non pharmacological Treatment.
- Pharmacological Treatment.
- Traditional Medicines.

Non Pharmacological Treatment:

Sleep hygiene

The concept of sleep hygiene refers to practices, habits, and environmental factors that are important for getting sound sleep. The four general areas important to sleep hygiene are the circadian rhythm (24-hour cycle); aging; psychological stressors that cause mini-awakenings (in which the brain wakes up for just a few seconds); and substances such as nicotine, caffeine, and alcohol.

Cognitive behavioral therapy

In this therapy, patients are taught improved sleep habits and relieved of counter-productive assumptions about sleep. Common misconceptions and expectations that can be modified Cognitive therapy can give people the proper information about sleep norms, age-related sleep changes, reasonable sleep goals, and the influence of naps and exercise.

Sleep restriction therapy

Sleep restriction therapy is based on the belief that excess time in bed makes sleep problems worse. SRT consists of limiting a person's time in bed to only that time when he or she is sleeping.

Music therapy

Research suggests that music therapy can improve sleep quality in acute and chronic sleep disorders. Music therapy did improve sleep quality in subjects with acute or chronic sleep disorders, however only when tested subjectively. Although these results are not fully conclusive and more research should be conducted, it still provides evidence that music therapy can be an effective treatment for sleep disorders.

Lifestyle Changes

Lifestyle adjustments can greatly improve your quality of sleep, especially when they're done along with medical treatments. Incorporating more vegetables and fish into your diet, and reducing sugar intake. Limiting your caffeine intake, especially in the late afternoon or evening. Decreasing tobacco and alcohol use. Eating smaller low carbohydrate meals before bedtime.

Pharmacological treatment

Several drugs are discovered for the treatment of sleep disorders. Several classification of drugs are present on based on their activity and chemical structure.

Sedative:

A drug that subdues excitement and calms the subject without inducing, though drowsiness may be produced. Sedation refers to the decreased responsiveness to any level of stimulation; is associated with some decrease in motor activity and ideation. The subject doesn't loses consciousness in sedation.

Hypnotics:

A drug that induces and/or maintains sleep, similar to normal arousable sleep. Hypnotics results in loss of consciousness. Thus it cannot be confused with "hypnosis", which refers to a trans-like state in which subject becomes highly passive and suggestible.

Both of these drugs are used as CNS depressants. Sedative and hypnotic drugs can be used for various sleep disorders such as Insomnia, Narcolepsy, Parasomnia, Sleepwalking, Night terror, etc,

Drugs used to treat Sleep Disorders

- **Benzodiazepines-** Diazepam, Midazolam, Nitrazepam, Alprazolam, etc.
- **Barbiturates**- Phenobarbital, Pentobarbital, Thiopental, Amobarbital, etc.
- **Non benzodiazepines**- Zolpidem, Alpidem, Saripidem, Ocinaplon, , Suproclone, etc.
- **Diphenylmethane-** Hydroxyzine
- **Azaspirodeconediones-** Buspirone , Gepirone, Ipsapirone, Tandospirone, etc.
- **Beta adrenoceptor antagonist-** Propranolol.
- **Carbamates-** Ethylcamate , Meprobamate , Carisoprodol, Phenprobamate , Mebutamate, etc.
- **Beta Carbolines-** Abecarnil , Gedocarnil.
- **Dibenzo – bicycle – octadiene -** Benzoctamine.
- **MonoAmine Oxidase Inhibitors-** Phenelzine.
- **Hormone**- Melatonin.

Traditional Medicines[26-28]:

Many plants are traditionally used by natives for diseases before the evolution of modern medicinal system. Siddha, Ayurveda, Unani, Homeopathy, Tribal medicine, Chinese traditional medicine and many more traditional system utilize local flora and fauna to treat various diseases and are effective yet to be scientifically proven. Few such medicinal plants are listed below.

Table. 1 Traditional plants used as Sedative and Hypnotic drugs

S.No	Herbal plants used as Sedative and Hypnotic
1.	*Myristrica fragrans*
2.	*Anamarita cocculus*
3.	*Elettaria cardamomum*
4.	*Amomum subulatum*
5.	*Aconitum ferox*
6.	*Passiflora incarnate*
7.	*Lavandula angustifolia*
8.	*Valeriana officinalis*
9.	*Scoparia dulcis*
10.	*Papaver somniferum*
11.	*Nepeta cataria*
12.	*Rawolfia serpentine*
13.	*Withania somnifera*
14.	*Cannabis sativa*
15.	*Melissa officinalis*

PLANT PROFILE

2. PLANT PROFILE

PLANT INTRODUCTION

Myristica malabarica is commonly called as Malabar nutmeg or kaatuhjathi. It is native to India found widely in western ghats hills. *Myristica malabarica* seed and seed aril is used as spice in Indian foods. They enhance the taste and aromatic flavor of the food[29]. Recent scientific studies proved their biological activity according to their traditional claims. They are now known to possess gastroprotective, antipromastigote, antioxidant, antifungal, nematicidal, antiproliferative, leukemic and solid tumor.

COMMON NAME AND TAXONOMY[30]

PLANT NAME

Myristica malabarica Lam.,

COMMON NAME

Malabar nutmeg.

False nutmeg.

Bombay mace.

SYNONYMS

Myristica dactyloides Wall.,

Myristica notha Wall.,

Myristica tomentosa J.Grah.,

Palala malabarica Kuntze.,

VERNACULAR NAMES

Hindi : Ramptri, Bambay-jayphal.

Kannada : Kanage, Doddajajikai.

Malayalam : Ponnampoovu, Kottappannu, Pathiripoovu, Panampalka.

Sanskrit : Bandhukapushpa, Gostani.

Tamil : Colaivenkai, Kattujatikkai.

Telugu : Adavijaikaya, Adividzajikaya.

TAXONOMICAL STATUS[31]

Kingdom : Plantae

Super division : Angiosperms

Phylum : Tracheophyta

Class : Mangoliopsida

Order : Mangoliales

Family : Myristicaceae

Genus : Myristica

Species : *Myristica malabarica* Lam.,

PLANT DESCRIPTION[32]

Myristica malabarica Lam. (Myristicaceae) is a perennial tree about 25m tall. It is endemic to India and found widely distributed in Western Ghats forest region. Now it is a vulnerable species according to IUCN red list. Hence it must be preserved.

HABIT AND HABITAT[33]

It is a large 15-25 mts tall perennial tree found evergreen forests upto 800mts. It is a swamp and lowland forest habitat tree. Large trunks with greyish black color. Flowering and fruiting season starts at feb-aug month. It is vulnerable species listed according to ICUN list due to drainage of swamp forests for agricultural purposes

Morphology

- **Height**: 25 m tall.

Fig. 4 Whole Tree of M.M

- **Trunk & Bark**: Bark greenish-black, smooth, blaze reddish

Fig. 5 Trunk and Bark of M.M

- **Branches and branchlets**: Branches- horizontal, Branchlets- subtree, glabrous.

Fig. 6 Branches of M.M

- **Exaduates**: Sap red from cut end of bark, profuse.

Fig. 7 Exudates of M.M

- **Leaves**: Leaves simple, alternate, distichous; Petiole 1-1.5cm long,caniculate above glabrous, lamina 9.5-22*3.7-10 cm, elliptic or elliptic oblong, apex acute or sub-acute, base acuteor attenuate, margin entire, glossy above, glabrous and glaucous beneath, coriaceous; mid rib raised above.

Fig. 8 Leaves of M.M

- **Inflorescence / Flower**: Flowers unisexual, uroceolate, white; male flower numerous in number and smaller than female flowers, in auxillary cymes; female flowers in umbels, 5-6 flowered.

Fig. 9 Flowers of M.M

- **Fruit and seed**: Capsule 5-7.5*1.8-3.5, oblong, pubescent; seed one, Aril covering the seed yellow and laciniate.

Fig. 10 Fruit of M.M

ETHANO MEDICINAL USES[34]

The plant *Myristica malabarica* is traditionally used as medicine and spices in food. The aril is used as febrifuge, cooling, expectorant. In Ayurveda, aril is used for many conditions related to vata such as, fever, bronchitis, cough and burning sensation. The fat extracted from seed is used to treat indolent ulcers, analgesics and for rheumatism. In Ayurveda, for disorders in vata seed fat is used for myalgia, sprains and sores. The plant is also used for anti-inflammatory, Analgesic, anti-ulcer, sedative, hypnotic, and antimicrobial actions.

PHYTOCHEMICAL CONSTITUENTS[35]

The plant *Myristica malabarica* was found to contain various phytoconstituents such as Isoflavones, Diarylnonanoids which consists of Malabaricones A-D, tannins and several other phytochemicals are also considered to be present in the plant. A brief study of phytoconstituents is needed for this plant.

Isoflavones

The heartwood of *Myristica malabarica* is found to have isolation of several isoflavones such as 7,4'-dimethoxy-5-hydroxyisoflavone , biochanin a, prunetin and a rare alpha-hydroxydihydrochalcone.

Diarylnanoids

The plant is found to consist of different type of diarylnonanoids which are commonly called as Malabaricones. *Myristica malabarica* contains four Malabaricones A, B, C and D. These malabaricones proved pharmacologically for various activity.

Tannins

From the investigation of the heart wood a tannin 1, 3-diarylpropanol is extracted.

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

A thorough literature survey was carried out in-order to identify the plant which is traditionally used for sedative and hypnotic purpose. *Myristrica malabarica* Lam.. was selected for the present study because it is traditionally used for sedative and hypnotic activity but not yet proven scientifically[36].

Literature review of *Myristrica malabarica* Lam.. was carried out to find out the research work on this plant. The review of literature showed that many studies were done on different part of this plant. This review is made to confirm that sedative and hypnotic activity on the seed aril was not yet proven scientifically. Hence further studies is to be carried out.

2.1. PHARMACOGNOSTICAL REVIEW

- Nagaraju S.K *et al.,* 2013 studied Ecological niche models (ENM) model predictions on adaptation of *Myristica malabarica* Lam., an endemic tree in the Western Ghats, India[37].
- R. Indra Iyer G. *et al.,* 2009 studied direct somatic embryogenesis in *Myristica malabarica* Lam.,[38]

2.2. PHYTOCHEMICAL REVIEW

- Bauri A.K *et al.,* 2016 studied crystal structure of an aryl cyclo-hexyl nona-noid. Isolated from the spice *Myristica malabarica*[39].
- Pandey R *et al.,* 2016 studied Rapid screening and quantitative determination of bioactive compounds from fruit extracts of Myristica species[40].
- Talukdar AC *et al.,* 2000 studied phytochemical investigation of the heartwood of *Myristica malabarica* and isolated an isoflavone[41].
- Purushothaman KK *et al.,* 1977 studied Malabaricones A--D, novel diarylnonanoids isolated from *Myristica malabarica.,* Lam[42]

2.3 PHARMACOLOGICAL REVIEW

- Pandey R *et al.,* 2016 studied Rapid screening and quantitative determination of *in-vitro* antiproliferative activity[43].
- Manna A *et al.,* 2015 studied The variable chemotherapeutic response of Malabaricone-A in leukemic and solid tumor cell lines depends on the degree of redox imbalance[44].
- Manna A *et al .,* 2015 studied generation of redox imbalance mediates the cytotoxic effect of malabaricone-a in a multidrug resistant cell line[45].
- Patil SB *et al.,* 2011 studied Insulin secretagogue, alpha-glucosidase and antioxidant activity of some selected spices in streptozotocin-induced diabetic rats[46].
- B.K. Manjunatha *et al.,* 2011 studied antioxidant and hepato protective effect of *Myristica malabarica* seed aril extracts on carbon tetrachloride induced hepatic damage[47].
- Maity .B *et al.,* 2009 studied Regulation of arginase/nitric oxide synthesis axis via cytokine balance contributes to the healing action of malabaricone B against indomethacin-induced gastric ulceration in mice[48].
- Maity .B *et al.,* 2008 studied *Myristica malabarica* heals stomach ulceration by increasing prostaglandin synthesis and angiogenesis[49].
- Nam Hee Choi et al., 2008 studied Nematicidal activity of malabaricones isolated from *Myristica malabarica* fruit rinds against *Bursaphelenchus xylophilus*[50].
- Sen R *et al.,* 2007 studied Antipromastigote activity of the malabaricones of *Myristica malabarica* (rampatri)[51].
- Patro BS *et al .,* 2005 studied Antioxidant activity of Myristica malabarica extracts and their constituents[52].
- Rani P *et al.,* 2004 studied Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant Salmonella typhi[54].

RATIONALE FOR SELECTION

4. RATIONALE FOR SELECTION

Plants are used as food and as well as medicine in our India. Many plants have been traditionally used in Siddha and Ayurveda medicinal systems to treat various disease and disorders. Although these plants are stated to have medicinal properties but they lack in detailed scientific studies. In a desire to bring value and recognition to our traditional medicinal system.

I have taken this plant *Myristica malabarica* Lam,. Belonging to the family Myristicaceae for the Sedative and Hypnotic studies. This plant is claimed in Ayurveda to possess sedative and hypnotic activity and used in formulation as one of the ingredient in Vata treatment which is one of the tri dhosha theoy.

Only a handful of studies are done in the plant *Myristica malabarica.* Lack of Pharmacognostical, Phytochemical and Pharmacological studies makes this plant an ideal target for the scientific studies of traditionally used medicinal plants. This plant is less studied and has a great potential in medicinal properties.

- No pharmacognostical work is done in the seed aril of this plant.
- No study for Sedative and Hypnotic activity is done in any part of this plant.
- This plant is claimed to have Sedative and Hypnotic activity in traditional medicinal system.
- Hence, the plant seed aril can be taken for the Sedative and Hypnotic activity studies.

AIM

&

OBJECTIVE

5. AIM AND OBJECTIVE OF THE STUDY

5.1 AIM

- To evaluate Pharmacognostical, Phytochemical, Sedative and Hypnotic activity on the seed aril of the plant *Myristica malabarica* Lam., .

5.2 OBJECTIVE

Pharmacognostical Studies

- To perform Macroscopical, Microscopical, Qualitative and Quantitative estimation of various Physico Phemical constituents of the seed aril.
- Thus to establish a Pharmacogonostical profile.

Phytochemical Studies

- To perform Preliminary Photochemical Screening of powder and plant extracts.
- Estimation of various Phytochemical Constituents through analytical method.
- Isolation of Active Constituents in the seed aril by Column Chromatography.
- Analysis of the Isolated Constituents by TLC, IR and FTIR spectroscopy.
- Elucidation of the Isolated Constituents by GC-MS.

Pharmacological Studies

- *In-vitro* Anti-Oxidant activity is used to determine the presence active constituents present in various extracts.
- *In-vivo* Spontaneous Locomotor Activity is to be done by Actophotometer for Sedative activity.
- *In-vivo* Potentiation of Hexobarbital Induced Sleep Time is to be done for Hypnotic activity.

PLAN OF STUDY

6. PLAN OF STUDY

6.1 COLLECTION OF PLANT STUDY

6.2 AUTHENTICATION OF THE PLANT

6.3 PHARMACOGNOSTICAL WORK

- **Macroscopical Studies**
- **Microscopical Studies**
  - **Powder microscopy**
  - **Histochemical study**
  - **T.S of seed aril**
  - **Physico chemical constants**

6.4 PHYTOCHEMICAL STUDIES

- **Preparation of extracts**
- **Preliminary phytochemical analysis**
- **Quantitative estimation of phytoconstituents**
- **Fluorescence analysis**
- **Thin layer chromatography**
- **High performance thin layer chromatography**

6.5 PHARMACOLOGICAL STUDIES

- **In-vitro antioxidant study**
  - **DPPH assay**
  - **H_2O_2 scavenging assay**
- **In-vivo study**
  - **Spontaneous Locomotor Activity by Actophotometer for Sedative activity**
  - **Potentiation of Hexobarbital Induced Sleep Time for Hypnotic activity**

6.6 ISOLATION OF ACTIVE CONSTITUENTS

- **Isolation of active constituents by column chromatography**
- **Analysis of Isolate constituents TLC, IR, FTIR and GCMS**

MATERIALS & METHOD

7. MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTICATION

Plant was collected from Theni district, Tamil Nadu, authenticated by P. Sathiyarajeswaran, Assistant director of Siddha Central Reasearch Institute, Arumbakkam, Chennai, as the seed aril of fruit *Myristica malabarica* Lam., The seed aril were shade dried, powdered and was stored in the air tight container and a portion of it is used for the Pharmacognostical and Phytochemical studies.

7.1 PHARMACOGNOSTICAL STUDIES

Pharmacognostical study is the preliminary step in the standardization of crude drugs. It primarily deals with the identification authentication and standardization of herbal medicinal plants through organoleptic character, histological character, powder microscopy, quantitative microscopy, histochemical analysis and Physiochemical observations as prescribed by an authoritative source such as World Health Organization[53-54].

Evaluation of crude drug involves determination of authenticity, quality, purity, potency, safety, efficacy, reliability and reproducibility of the evaluation results for varying batch of crude drug. This is also used in the detection of nature of adulteration. Systematic identification of crude drugs and their quality assurance gives an integral part of drug description.

MACROSCOPICAL EVALVATION

Macroscopical character includes organoleptic characters and morphological features of the plant were studied. Hence, this observation is of primary importance before any further testing can be carried out. Organoleptic characters like color, odor, taste and nature are studied. Morphological characters like size, shape, surface, fracture and thickness were observed for the determination of safety, efficacy and purity of crude drugs[55].

MICROSCOPIC EVALUATION

Staining method

Fixation of plant organ – The sample or seed was cut fixed in FAA solution (Formalin 5ml + Acetic acid 5ml + 90ml of 70% Ethanol). The specimen was dehydrated after 24 hours of fixing. The seeds were graded with series of tertiary butyl alcohol, as per the standard method[56].

Infiltration of the specimen - It was carried out by gradual addition of 58 – 60º C of melting pointed paraffin wax until Tertiary butyl alcohol (TBA) solution attained super saturation. The specimens were cast into paraffin blocks[57].

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10 - 12µg. Dewaxing of the sections were done by customary procedures. The sections were stained with Toludine blue. Since toludine blue, a polychromatic stain. The staining results were remarkably good. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to subrein, violet to mucilage and blue to the protein bodies. Whenever necessary sections were also stained with safranin, fast green and iodine for starch[58].

PHOTO MICROGRAPHS

Microscopic descriptions of tissues were supplemented with photo micrographs whenever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations bright field was used.

For the study of crystals and lignified cells, polarized light was employed. Since, these structures have birefringent property under polarized light they appear bright against dark background. Descriptive terms of the anatomical features are as given in the standard anatomy books.

POWDER MICROSCOPY[59]

The shade dried, powdered plant material was used for powder microscopic analysis. The organoleptic characters were observed and to identify the different characteristic features various staining reagent were used. Powder was stained with 1% phloroglucinol in 90% ethanol, concentrated hydrochloric acid and observed through microscope. All the lignified cells stained with pink color. Calcium oxalate crystals were observed under the polarized light microscope.

HISTOCHEMICAL STUDIES[60]

Portions of seed aril of the plant *Myristica malabarica* were used. The peels were soaked in water before taking the sections. The sections were stained using specific reagents (N/50 iodine, dilute ferric chloride, phloroglucinol and con.hydrochloric acid, picric acid, ortho toludine blue and dragendroff reagent) to observe and locate starch, lignin, tannin, protein, flavonoid and alkaloid respectively as per the protocols. The stained sections were then washed in water to remove the excess stain and observed under a microscope(Olympus BX41).Pictures of the sections were taken by DP71 fixed with Olympus BX41microscope and processed by using the software image pro express 6.0.

PHYSICO-CHEMICAL CONSTANTS[61-63]

Shade dried powdered plant materials of the fruits of *Myristica malabarica* was used for the determination of the Physiochemical constants in accordance with the WHO guidelines.

DETRMINATION OF ASH VALUES

Ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The residue remaining after incarnation is the ash content of the drug, which simply represents inorganic salts, naturally occurring drug or adhering to it or deliberately added to it, as a form of adulteration.

Ash value of a crude drug is defined as the inorganic residue remaining after incineration, which complies of inorganic salts, naturally occurring in drug or adhering to

it or deliberately added to it as a form of adulteration. Hence it is used for the determination of the quality and purity of the crude drug in the powdered form.

TOTAL ASH:

Total ash method is designed to measure the total amount of material remaining after ignition. They include both physiological ash which is derived from plant tissue itself and non-physiological ash which is the residue of extraneous matter adhering to the plant surface.

Procedure:

Silica crucible was heated to red hot for 30 minutes and cooled in the desiccators Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tarred silica dish at a temperature not exceeding 450^0c until the sample is free from carbon, cooled in desiccators and weighed. The ash obtained was weighed. The percentage of total ash was calculated.

%Total Ash value= (Wt of total ash/ wt of total drug taken) x 100

WATER SOLUBLE ASH:

The difference in weight between the total ash and the residue after treatment of the total ash in water.

Procedure:

Total ash obtained is boiled for 5 minutes with 25 ml of water, insoluble matter were collected in an ash less filter paper, washed with hot water and ignite for 15 min at a temperature not exceeding 450^0. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per gram of air-dried material.

%Water soluble ash = (Weight of residue obtained/ Weight of the sample taken) X 100

ACID INSOLUBLE ASH:

The residue obtained after boiling the total ash with dilute hydrochloric acid, the remaining insoluble matters are ignited and measured. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure:

To the crucible containing total ash of the sample, 25 ml of dilute hydrochloric acid is added. The insoluble matter is collected on an ash less filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Filter paper containing the insoluble matter to the original crucible, dry on hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccators for 30 minutes and weighed without delay. Content of acid-insoluble ash with reference to the air dried drug is calculated.

%Acid insoluble ash = (Weight of the residue obtained/ Weight of the sample taken) X 100

SULPHATED ASH

Sulphated ash test is used to measure the amount of residual substance not volatilized from a sample. These test are usually used to determine the content of inorganic substance.

Procedure:

Silica crucible are heated to redness for 10 minutes, allowed to cool in a desiccator and weigh. 2 g of sample were accurately weighed, ignited gently then thoroughly charred. Cool, moistened the residue with 1 ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800 ± 25^{o} until all black particles have disappeared. Crucible are allowed to cool, add few drops of sulphuric acid and heat. Ignite as before, allow to cool and weigh. This process is repeated until two successive weighing differ by more than 0.5 mg.

%Sulphated ash = (weight of the residue obtained/ Weight of the sample taken) X 100

DETERMINATION OF EXTRACTIVE VALUES:

Extractive values are useful for the evaluation of Phytoconstituents especially when the constituents of a drug cannot be readily estimated by any other means. Further these values indicate the nature of the active constituents present in a crude drug.

Determination of water soluble extractive

5gm of air dried coarsely powdered sample was weighed and macerated with 100ml of chloroform water (95ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for rest eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and was dried at 105°C for 1 hour in the hot air oven and cooled in desiccators for 30min and weighed. The process was repeated till a constant weight was obtained; the percentage of water soluble extractive value was calculated with reference to the air dried drug.

%Water soluble extractive value = (wt of the dried extract/ wt of the sample taken) X 100

Determination of alcohol soluble extractive

5gm of the coarsely powdered sample was weighed and macerated with 100ml 90% ethanol in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105°C for 1hour in a hot air oven. The dish was cooled in desiccator and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to the air dried drug was calculated.

% Alcohol soluble extractive = (wt of the dried extract/ wt of the sample taken) X 100

Determination of non-volatile ether soluble extractive (fixed oil content)

A suitably weighed quantity of the sample was transferred to an extraction thimble and extracted with solvent ether or petroleum ether (Boiling Point 40 - 60°C) in a Soxhlet for 6 hours. The extract was filtered into a tarred evaporating dish, evaporated and dried at 105°C to constant weight. The percentage of nonvolatile ether soluble extractive value with reference to the air dried drug was calculated.

%Nonvolatile ether soluble extractive= (wt of the dried extract/ wt of the sample) X 100

Determination of volatile ether soluble extractive

2gm of powdered sample was accurately weighed and extracted with anhydrous diethyl ether in a continuous extractive apparatus for 20hours. The ether solution was transferred to tarred porcelain dish and evaporated spontaneously, dried over phosphorous pent oxide for 18hours and the total ether extract was weighed. The extract was heated gradually and dried at 105°C to constant weight. The loss in weight represents the volatile portion of the extract.

Volatile ether soluble extractive= (wt of the dried extract/ wt of the sample taken) X 100

DETERMINATION OF MOISTURE CONTENT:

Loss on drying

10 g of the sample substances (without preliminary drying) was taken in a tarred evaporating dish. Use of high speed mill in preparing the samples are avoided. The sample in the tarred evaporating dish were placed in the drying chamber (105°C) for 5 hours and weigh. Drying and weighing is continued every one hour interval until the difference between the two successive weights is not more than 0.25 percent. Constant weight is reached when the two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, show not more than 0.001 g difference. Percentage moisture content is compared with respect to the air dried sample.

% Moisture content = (Final weight of the sample/ Initial weight of the sample) X 100

Determination of foaming index

1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml boiling water. The flask was maintained at temperature 80-90°C for about 30min. It was then cooled and filtered into a volumetric flask and sufficient water was added through the filtrate to make up the volume to 100ml. The decoction was

poured into 10 stopper test tube (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml, 3ml, 4ml up to 10ml and the volume of the liquid in each tube was adjusted with water to 10ml. The tubes were stoppered and shaken in a length wise motion for 15 seconds, two shakes per second. Allowed to stand for 15min and the height of the foam was measured. The results are assessed as follows.

If the height of the foam in every tube is less than 1cm, the foaming index is less than 100. If a height of 1cm is measured in any tube, the volume of the plant material decoction in the tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

If the height of the foam is more than 1cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilution of the decoction in order to obtain a result. Calculate the foaming index using the following formula:

$$\text{Foaming index} = 1000/a$$

Where, a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

Determination of swelling index

The swelling index is the volume in ml occupied by the swelling of 1gm of plant material under specified conditions. A specified quantity of the plant material were previously reduced to the required fineness was accurately weighed and transferred into a 25ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125mm, marked in 0.2ml divisions from 0 to 25ml in an upward direction. Unless otherwise indicated in the test procedure, add 25ml of water and shake the mixture thoroughly every 10min for 1hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material was measured including any sticky mucilage. Calculate the mean value of the individual determination, related to 1gm of plant material.

DETERMINATION OF VOLATILE OIL IN THE DRUG:

Determination of the volatile oil in a sample is done by distilling the drug with a mixture of water and glycerine, collecting the distillate in a graduated tube in which the aqueous portion is automatically separated and returned to the flask and the volume of oil collected is measured.

The content of the oil is expressed as percentage v/w. and it is performed in the clevenger's apparatus.

Procedure:

Weighed amount of sample is distilled with the 75 ml of glycerine and 175 ml of water in the one liter distilling flask with few pieces of earthen ware and one filter paper 15 cm cut into small head. Condenser is placed above the flask and water is allowed to run through the condenser. Heating is discontinued after 3 to 4 hours. Volume of oil collected is read in the graduated tube. The measured yield of volatile oil is taken to be the content of volatile oil in the drug.

Percentage of volatile content = (Amount of volatile oil obtained/ Weight of drug) X 100

QUALITATIVE AND QUANTITATIVE ESTIMATION OF HEAVY METALS AND INORGANIC ELEMENTS[64]

Plant minerals play a vital role in metabolism. Presence of elements vary with the soil, climate conditions etc. There are essential and non-essential elements which may be beneficial or harmful to living things. Non-essential elements like lead, arsenic, cyanide, chromium, cadmium, aluminium and silver bring about toxic effects resulting in intoxication. Hence, qualitative and quantitative estimation of inorganic elements in the plant *Myristrica malabarica* Lam., were carried out.

QUALITATIVE ANALYSIS OF INORGANIC ELEMENTS AND HEAVY METALS

To the ash of the drug material 50%v/v hydrochloric acid was added and kept for 1 hour. It was filtered and the filtrate was used for the following tests.

- **Aluminium**: White gelatinous precipitate of aluminium hydroxide is formed on addition of ammonia solution. It is slightly soluble in excess of the reagent. The precipitate dissolves readily in strong acid and base, but after boiling it becomes insoluble.
- **Arsenic:** Arsenious salts in neutral solution react with solution of copper sulphate to form green precipitate (scheele's green) which on boiling gives a red precipitate of cupric oxide.
- **Borate:** The mixture obtained by the addition of sulpuric acid and alcohol (95%) to a borate when ignited, burns with flame tinged with green.
- **Calcium:** Solution of calcium salts, when treated with ammonium carbonate solution, yield a white precipitate after boiling and cooling the mixture (it is insoluble in ammonium-chloride solution).
- **Carbonate**: Carbonate, when treated with dilute acid effervescence, liberating carbon-dioxide which is colourless and produces a white precipitate in calcium hydroxide solution.
- **Chlorides**: Chlorides, when treated with silver nitrate solution, yield a white crude precipitate which is insoluble in nitric acid, but soluble after being well washed with water, in diluted ammonia, from which it is re precipitated by the addition of nitric acid.
- **Copper**: An excess of ammonia, added to a solution of a cupric salt, produces first a bluish precipitate and then a deep blue coloured solution.
- **Iron**: Solution of ferric salts, when treated with potassium ferrocyanide solution, yields an intense blue precipitate which is insoluble in dilute HCl.
- **Lead**: Strong solution of lead salts, when treated with HCL, yield a white precipitate. Which is soluble in boiling water and is re deposited as crystals when the solution is cooled.
- **Magnesium:** Solution of magnesium salts, when treated with ammonium carbonate solution and boiled, yield a white precipitate, but yield no precipitate in the presence of ammonium chloride solution.

- **Mercury:** Solution of mercury salts, when treated with sodium hydroxide solution, yields a yellow precipitate.
- **Nitrate**: With solution of ferrous sulphate no brown colour was observed but if sulphuric acid is added (slow from the side of the test tube), a brown colour is produced at the junction of two liquids, indicating the presence of nitrates.
- **Phosphate**: Solution of phosphate when treated with silver nitrate with dilute ammonia solution and in dilute nitric acid yield yellow precipitate of normal silver ortho phosphate (distinction from meta and pyrophosphate) solution.
- **Potassium**: Moderately strong potassium salts, which have been previously ignited to remove ammonium salts, when treated with perchloric acid (60%) yield a white crystalline precipitate.
- **Silver**: Solution of silver salts, when treated with potassium iodide solution yield a cream coloured precipitate which is insoluble in dilute ammonia solution and in nitric acid.
- **Sulphates**: Solution of sulphates, when treated with lead acetate solution yields a white precipitate which is insoluble in ammonium acetate solution and in sodium hydroxide.

QUANTITATIVE ESTIMATION OF INORGANIC ELEMENTS[65]

Inductive coupled plasma-Optical emission spectroscopy (ICP-OES)

It is an excellent multi-element technique with relatively good sensitivity and selectivity when configured correctly. This technique utilizes the plasma as an ion source or light emission source are capable of producing values.

QUANTITATIVE ANALYSIS OF HEAVY METALS

Instrumentation parameters:

Instrument name: Inductive coupled plasma-Optical emission spectroscopy

Instrument Model: PE Optima 5300DV ICP-OES; Optical system Dual view-axial or radial

Detector system: Charge coupled detector, (UV-Visible detector which is maintaining at -40º C) to detect the intensity of the emission line.

Light source (Torch): Positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a simple software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Standard alumina injector: 2.0mm inner diameter.

Spray chamber: Scott type

Nebulizer: Cross flow gem tip.

Preparation of sample by acid digestion method:

50mg of powder was treated with acid mixture of sulphuric acid: water in the ratio of 4:1in the Kjeldahl flask and heated continuously till the solution is colorless. The sample mixture was then transferred in a 25ml volumetric flask and made up to the volume with distilled water. Blank solution was prepared as above without sample.

The standards of Arsenic, Lead, Mercury and Cadmium were prepared as per the protocol and the calibration curve was developed for each of them.

Detection:

Samples were analyzed for the detection and quantification of the calcium, sulphate, borate, silver, aluminum, copper, potassium, chloride by Inductively Coupled Plasma Emission Spectrometry.

7.2 PHYTOCHEMICAL STUDIES

PRILIMNARY PHYTOCHEMICAL SCREENING[66-68]

Phytochemical evaluation is used to determine the nature of Phytoconstituents present in the plant by using suitable chemical tests. It is essential to study the

pharmacological activities of the plant. It can be done by confirmation with different chromatographic techniques like TLC and HPTLC. Therefore a complete investigation is required to characterize the Phytoconstituents qualitatively and quantitatively.

Preparation of Extracts

Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites into the extracting solvent depends upon its polarity.

Extraction

The first step was the preparation of successive solvent extracts. The dried coarsely powdered sample of *Myristica malabarica* (500gm) was first extracted with Benzene (60-80°C) in Soxhlet apparatus and then with solvents of increasing polarity like ethanol at (60 - 70ºC). They were then followed with distillation by Clevenger's appartus. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were recorded and preceded for further detailed Phytochemical and pharmacological screening.

PRELIMINARY PHYTOCHEMICAL SCREENING

The chemical tests for various Phytoconstituents in the dried powder and extracts of *Myristica malabarica* Lam., were carried out as described below and the results were recorded.

1. **Detection of Alkaloids**

- Dragendorff's reagent :

  The substance was dissolved in 5ml of distilled water, to this 5ml of 2M HCL was added until an acid reactions occurs, then 1ml of Dragendorff's reagent was added and examined for an immediate formation of an orange red precipitate.

- Mayer's reagent:

  The substance was mixed with little amount of dilute hydrochloric acid and Mayer's reagent and examined for the formation of white precipitate.

➢ Wagner's reagent :

The test solution was mixed with Wagner's reagent and examined for the formation of reddish brown precipitate.

2. **Detection of Glycosides**

➢ Borntrager's test :

The powdered material was boiled with 1ml of sulphuric acid in a test tube for five minutes. Filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

➢ Modified Borntrager's test :

The test material was boiled with 2ml of the dilute sulphuric acid. This was treated with 2ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 minutes, and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

3. Detection of Steroids and Triterpenoids

➢ Libermann Burchards Test:

The powdered drug was treated with few drops of acetic anhydride, boiled and cooled. Conc.sulphuric acid was added from the sides of the test tube, brown ring is formed at the junction of two layers and upper layer turns green which shows presence of steroids and formation of deep red color indicates presence of tri terpenoids.

➢ Salkowski Test :

The extract was treated with few drops of concentrated sulphuric acid, red color at lower layer indicates presence of steroids and formation of yellow colored lower layer indicates presence of tri terpenoids

.

4. **Detection of Flavonoids**

- Shinoda test :

To the solution of extract, few piece of magnesium turnings and concentrated Hcl was added drop wise, pink to crimson red, occasionally green to blue color appears after few minutes indicates the presence of flavonoids.

- Alkaline reagent test :

To the test solution few drops of sodium hydroxide solution was added, intense yellow color is formed which turns to colorless on addition of few drops of dilute acid indicate presence of flavonoids.

5. **Detection of Carbohydrates**

- Molisch's test :

To the test solution few drops of alcoholic alpha napthol and few drops of conc. sulphuric acid were added through the sides of test tube, purple to violet color ring appears at junction.

- Fehling's test :

The test solution was mixed with Fehling's I and II and heated and examined for the appearance of red coloration for the presence of sugar.

6. **Detection of Phenols**

- Ferric chloride test :

A small quantity of substance were dissolved with 2ml distilled water and a few drops of 10% aqueous ferric chloride solution was added and observed for appearance of blue or green color.

7**. Detection of Proteins**

- Biuret test :

The sample was treated with 5-8 drops of 10% w/w copper sulphate solution, violet color is formed.

8. **Detection of Tannins**

- Lead acetate test :

  The test solution was mixed with basic lead acetate solution and examined for formation of a white precipitate.

- Ferric chloride test :

  A few drops of 5% aqueous ferric chloride solution was added to 2ml of an aqueous extract of the drug and examined for the appearance of bluish black color.

9. **Detection of Saponins**

- A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for 3 minutes. Development of any honey comb like froth was examined.

10. **Detection of Gum and Mucilage**

- Small quantities of test substances was dissolved in 5 to 10ml of acetic anhydride by means of heat, cooled and add 0.05ml of concentrated sulphuric acid; it is examined for the formation of bright purplish red color.

11. Detection of fixed oils and fats:

- Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils and fats.

FLUORESCENT ANALYSIS[69]

Fluorescence analysis was carried out in day light and in UV light. The leaf powder and extracts were treated with different solvents and the fluorescence was observed in day light and in near and far UV light.

QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT[70-74]

Total Flavanoid content

Total flavanoid content was determined by calorimetric method, using quercetin as a standard. The test samples were individually dissolved in DMSO. Then the sample solution (150 µl) was mixed with 150 µl of 2% aluminium chloride. After 10min of incubation at ambient temperature, the absorbance of the supernatant was measured at 435nm using spectrophotometer. Three replicates were made for each test sample. The total flavanoid content was expressed as quercetin equivalent in mg/gm extract (mg QRT/gm extract).

Total phenolic content (Folin – Ciocalteu's assay)

Total phenolic content of the extracts were determined using Folin –Ciocalteu's assay. 0.5ml extract solutions were mixed with 2.5ml of 10 fold diluted Folin Ciocalteu's reagent and 2.5ml of 7.5% sodium carbonate. After incubation at 40°c for 30 minutes, the absorbance of the reaction mixtures was measured at 765nm in a spectrophotometer. Three replicates were made for each test sample. Gallic acid was used as a standard and total phenolic content of the extract was expressed in mg of Gallic acid equivalents (mg GAE/g extract).

Total alkaloid content

Accurately measure Aliquots (.2, .4, .6, .8, 1.0 ml) atropine standard solution and transferred in to volumentric flask. To the standard 5ml of phosphate buffer and 5 ml of BCG solution were added. To the mixture 1, 2, 3, 4 and 5 ml of chloroform was added. The extracts were dissolved in chloroform and diluted accordingly. The solutions were viewed at 470 nm with chloroform as blank. Atropine sulphate is used as a standard and total alkaloid content was expressed in mg of atropine sulphate equivalents (mg AS/gmextract)

CHROMATOGRAPHY[75-77]

Chromatography methods are important analytical tool in the separation, identification and estimation of components present in the plant.

THIN LAYER CHROMATOGRAPHY

Principle

Thin layer chromatography is a technique used for the separation, identification and estimation of single or mixture of components present in the various extracts. It is reliable technique in which solute undergoes distribution between two phases, stationary and mobile phase. The separation is mainly based on the differential migration that occurs when a solvent flows along the thin layer of stationary phase. This may be achieved by partition and adsorption depending on stationary phase used.

TLC Plate Preparation

The plates were prepared using Stahl TLC spreader. 40gm of silica gel G was mixed with 85ml of water to prepare homogenous suspension and poured in the spreader. 0.25mm thickness of plates was prepared, air dried until the transparency of the layer disappeared, then dried at 110°C for 30 minutes and kept in desiccators.

Selection of mobile phase:

Solvent mixture was selected on the basis of the Phytoconstituents present in each extract. Factors such as nature of components, stationary phase, mobile phase, polarity, influence the rate of separation of constituents. From the vast analysis, best solvent was selected which showed good separation with maximum number of components.

HPTLC- FINGERPRINT PROFILE:

HPTLC is one of the versatile chromatographic method which helps in the identification of compounds and thereby authentication of purity of herbal drugs. The time required in this method for the demonstration of most of the characteristic constituents of a drug is very quick and short. In addition to qualitative detection, HPTLC also provides

semi-quantitative information on major active constituents of a drug, thus enabling an assessment of drug quality.

HPTLC serves as a convenient tool for finding the distribution pattern of Phytoconstituents which is unique to each plant. The fingerprint obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extract.

Instrument Conditions:

Sample used **:** Ethanol Extract

Instrument : CAMAG HPTLC

HPTLC Applicator : CAMAG LINOMAT IV

HPTLC Scanner : CAMAG TLC SCANNER II

Sample dilution : 100mg of sample extracted with 1ml of Ethyl acetate

Volume of injection : 20µl

Mobile phase : Hexane: Ethyl acetate: Chloroform: Toluene: Methanol: Formic Acid (2:2:1:4:0.5: 0.5)

Lambda max : 254nm

Lamp : Deuterium

Stationary phase : TLC silica gel 60 F254 (Merck)

Equipment:

A Camag HPTLC system equipped with a sample applicator Linomat IV, Twin trough plate development chamber, TLC Scanner II.

Chromatographic conditions:

The estimation has been done using the following chromatographic conditions. Chromatography was performed on a 10 × 10cm pre-activated HPTLC silicagel 60 F254

plate. Samples were applied to the plate as 6mm wide band with an automatic TLC applicator Linomat IV with nitrogen flow (CAMAG, Switzerland), 8mm from the bottom. Densitometric scanning was performed on CAMAG scanner II. The plates were pre-washed with solvent ethyl acetate.

7.3 PHARMACOLOGICAL STUDIES

7.3.1*IN VITRO* ANTI OXIDANT ACTIVITY[78-80]

There has been a lot of interest in the development of alternative medicine for sleep disorders. Oxidative stress plays a major role in the progression of sleep disorder. It occurs when the balances between free radicals which inhibits GABA binding activity and anti-oxidants helps in prevention of GABA binding inhibition.

Anti-oxidants like poly phenols and flavonoids are therefore very helpful in reduction of stress factors and free radical formation which inhibits GABA binding activity. It has become evident that flavonoids are able to exert enhancement of GABA binding activity even at low concentration[81-82].

In vitro antioxidant activities for the extracts were carried out using the following methods.

1. DPPH assay
2. Hydrogen peroxide scavenging assay.

DPPH Assay: 1, 2-DIPHENYL-2-PICRYL HYDRAZYL RADICAL (DPPH)

Initial volume 0.1 mL of various concentrations of samples was mixed with 0.4 mL of 0.3M DPPH reagent prepared in ethanol. The mixture was shaken thoroughly and incubated in the darkness at room temperature for 30 min. The absorbance of the reaction was measured spectrophotometrically at 517nm, immediately after mixing and then after incubation as well. The scavenging effect of DPPH free radical was calculated by using the following equation.

% scavenging activity = Abs (control) - Abs (standard) / Abs (control) × 100

Where control is the absorbance of the blank (a reaction with all the reagents except the test extract), and absorbance of sample is the absorbance of the test extract. Tests were carried out in triplicates to obtain 50% inhibition (IC50). Using Butylated hydroxy Toulene.

HYDROGEN PEROXIDE SCAVENGING ASSAY (HPSA)

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch. A solution of Hydrogen peroxide (2mmol/l\lt) was prepared in phosphate buffer (PH 7.4). Various concentrations of extracts (10-100µg/ml) were added to hydrogen peroxide solution (0.6ml). Absorbance at 230nm was determined after 10min against a blank solution containing phosphate buffer without hydrogen peroxide.

% scavenging activity = Abs (control) - Abs (standard) / Abs (control) × 100
Compared with the ascorbic acid standard.

7.3.2 *IN-VIVO* EVALUVATION SEDATIVE AND HYPNOTIC ACTIVITY

EXPERIMENTAL DESIGN:

Materials and Methods

Plant extract

Benzene, ethanol and aqueous extracts from seed aril of *Myristica malabarica* Lam.,

Animal selection and procurement[83]

Healthy young Swiss-Albino mice (weighing about 20-30gm) were procured from the Madras Medical College animal house. The animals used for the entire study was approved by the Institutional Animal Ethical Committee which is certified by the Committee for the purpose of control and supervision of experiments on animals, India.

Approved CPCSEA Registration No: IAEC/MMC/10/2016 Dated: 21/11/2016

The procured animals were kept in a clean, dry polycarbonate cages and maintained in a well-ventilated animal house. The temperature of experimental animal room was maintained at 22ºC (± 3ºC) and the relative humidity was maintained from 50-60%. Lighting was artificially maintained for 12hrs dark and 12hrs light. All the animals were kept in the cages for at least 5days prior to dosing for acclimatization to the laboratory conditions. The animals were fed with standard pellet diet and water was given ad libitum. Before starting the dose, the animals were fasted overnight but allowed to access water.

ACUTE TOXICITY STUDIES[84-85]

ACUTE ORAL TOXICITY STUDY (FIXED DOSE PROCEDURE) OECD 425 GUIDLINE FOR THE TESTING OF CHEMICALS

The organization of economic co – operation and development (OECD) guideline 425 was followed the acute oral toxic fixed dose method is a stepwise procedure with 5 rats of single sex per step (one animal per step). Depending upon the mortality and morbidity status of the animal, on average of 2 to 4 steps may be necessary to allow judgment on the acute oral toxicity of the substance. This procedure results in the use of minimal number of animal while allowing for acceptable data based scientific conclusion.

Literature survey showed that acute toxicity of the extracts was determined according to the OECD guideline No. 425 (20). Swiss albino mice of both sex weighing 27-30 g were used for Pet ether and Ethanolic Extract of *Myristica malabarica* Lam., was given to four groups (n = 5) of animals each at 5, 50, 300 and 2000 mg kg-1 b.w. p. o. The treated animals were under observation for 14 days, for mortality and general behaviour. No death was observed till the end of the study. The test sample was found to be safe up to the dose of 2000 mg/kg. So, $1/5^{th}$ (400mg) of the dose were selected for this study.

SEDATIVE ACTIVITY[86-88]

Evaluvation of Sedative Activity by Spontaneous Locomotor Activity Using Actophotometer.

Grouping of animals

Animals were divided into 5 groups of six animals each.

- Group I: Vehicle -2% tween 80 solution (2ml/kg b.wt) was orally administered.
- Group II: Standard drug [Diazepam] - (3 mg/kg b.wt) was orally administered.
- Group III: Test drug I [Benzene extract] - (400mg/kg) was orally administered.
- Group IV: Test drug II [Ethanolic extract] - (400mg/kg b.wt) was orally administered.
- Group V: Test drug III [Aqueous extract] - (400mg/kg b.wt) was orally administered.

After 30 mins of oral administration of drugs the animals were experimented for sedative activity using Actophotometer,

ACTOPHOTOMETER

It consists of six built in photo sensor and 4 digit digital counter to indicate the loco meter activity. It measures then spontaneous and indicated activity with digital totalize. It also incorporates electric shock of up to 100 volts for activating rats. The stimulus is variable from 0 to 100v & indicating on meter

Fig. 11 Actophotometer

Most of the CNS acting drugs influence the locomotor activities in man & animals. The CNS depressant drugs such as barbiturates & alcohol reduce the motor activity while the stimulants such as caffeine & amphetamines increase the activity. In other words, the locomotor activity can be an index of wakefulness (alertness) of mental activity.

The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photo cell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square arena in which the animal moves. Both rats & mice may be used for testing in this equipment.

HYPNOTIC ACTIVITY[89-92]

Evaluvation Of Hypnotic Activity By Potentiation Of Hexobarbital Induced Sleep Time.

Grouping of animals

Same group of animal is use for this experiment after washout period of 15 days for the given test drug to be completely excreted.

Animals were divided into 5 groups of six animals each.

- Group I: Vehicle -2% tween 80 solution (2ml/kg b.wt) was orally administered.

- Group II: Standard drug [Diazepam] - (3 mg/kg b.wt) was orally administered.
- Group III: Test drug I [Benzene extract] - (400mg/kg) was orally administered.
- Group IV: Test drug II [Ethanolic extract] - (400mg/kg b.wt) was orally administered.
- Group V: Test drug III [Aqueous extract] - (400mg/kg b.wt) was orally administered.

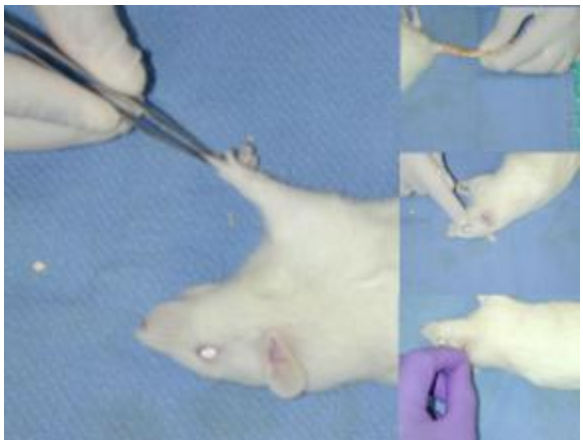


Fig. 12 : Righting reflex

All groups of animals will be administered with Hexobarbital 50mg/kg, i.p. after 1 hour of standard and test drug administration.

Writhing reflex is an indicator of mice wakefulness or conscious. This parameter is evaluvated for this activity and the extension of sleep time caused by the drug is noted and reported for hypnotic activity.

Potentiation of hexobarbital sleeping time

Thirty min after i.p. or s.c. injection or 60 min after oral dosing 60 mg/kg hexobarbital is injected intravenously. The animals are placed on their backs on a warmed (37oC) pad and the duration of loss of the righting reflex (starting at the time of hexobarbital injection) is measured until they regain their righting reflexes. Injection of 60 mg/kg hexobarbital usually causes anesthesia for about 15 min. If there is any doubt as to

the reappearance of the righting reflex, the subject is placed gently on its back again and, if it rights itself within one minute, this time is considered as the endpoint. The test and standard drugs are given prior 1hr orally before i.p administration of hexobarbital. The extension of time taken for righting reflex is noted for various drugs.

STATISTICAL ANALYSIS[93-95]

The statistical analysis was carried by one way ANOVA followed by Dunnet's —t test. P values <0.05 (95% confidence limit) was considered statistically significant, using Software Graph pad Prism 6.0

7.4 ISOLATION AND CHARACTERISATION OF PHYTOCONSTITUENTS BY COLUMN CHROMATOGRAPHY

Column chromatography is an isolation technique in which phytoconstituents are being eluted by adsorption. The principle involved in this separation of constituents is adsorption at the interface between solid-liquid. The component must have various degree of affinity towards adsorbent and also reversible interaction to achieve successful separation. No two compounds are alike in above respect. Low affinity compound will elute first. Adsorbent used is silica gel.

Ethanolic extract of *Myristica malabarica* was found to possess maximum phytoconstituents with potent sedative and hypnotic activity. An attempt was made to fractionate the ethanolic extract by column chromatography.

COLUMN CHROMATOGRAPHY[96]:

Type of extract : Ethanolic extract

Method : Dry packing method

Packing material : Silica gel G70-325

PROCEDURE:

The ethanolic extract was subjected to Silica gel column chromatography for isolation of phytoconstituents.

An appropriate column sized 2.5 cm diameter and 60 cm length was used. It was washed with water and rinsed with acetone and dried completely. Little of pure cotton was placed at the bottom of column with help of a big glass rod. Solvent Hexane was poured in to the column 3/4th level. Before packing, ethanolic extract 10 gm was diluted with same solvent and thoroughly mixed with 30 g of graded silica gel. It was constantly mixed until it became free flow. When it reached at defined state it was slowly poured in to the column containing hexane solvent with slight movement of stirring by glass rod to avoid clogging. Once it got settled, little cotton was placed on top of silica gel extract mixture pack to get neat column pack. The knob at bottom was slowly opened to release the solvent. The elute was done using hexane, followed by hexne-ethyl acetate mixture and ethyl acetate-ethanol mixture. Each fraction were collected and analyzed quantitatively. Similar fractions were mixed together, allowed to evaporate to remove solvent.

TLC[97]

As soon as the fraction eluted, it was analyzed by using readymade TLC plate with suitable mobile solvent according to the polarity of elute.

SOLVENT

Methanol: Chloroform in 1: 9 ratio.

OBSERVATION

The developed chromatogram was observed under UV, Fluorescence and also derivatised using detecting agent.

CHARACTERISATION OF ISOLATED COMPOUND

PHYSICAL EVALUATION:

1. Physical properties of the isolated compounds are evaluated, such as

    - Colour
    - Nature
    - Solubility
    - Molecular weight

- Molecular formula
- Melting point
- Boiling point

2. Further the isolated compounds were characterized by following Spectroscopic methods. Such as

- IR Spectroscopy
- Mass spectroscopy (GCMS)

IR SPECTROSCOPY[98]

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic chemists. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. IR spectroscopy is an important and popular tool for structural elucidation and compound identification. The possible characteristic bands of the nucleus are

1. 3540-3300 cm-1 N-H Stretching Vibration
2. 3670-3230 cm-1 O-H Stretching Vibration
3. 1690-1630 cm-1 C=N Stretching Vibration
4. 2975-2840 cm-1 C-H Aliphatic Stretching Vibration
5. 3100-3000 cm-1 C-H Aromatic Stretching Vibration

MASS SPECTROSCOPY[100-101]

Mass Spectrometry is an analytic technique that utilizes the degree of deflection of charged particles by a magnetic field to find the relative masses of molecular ions and fragments.

- ✓ Determining molecular mass
- ✓ Finding out the structure of an unknown substance
- ✓ Verifying the identity and purity of a known substance
- ✓ Providing data on isotopic abundance.

GC-MS[102]:

It is a combined technique, used for molecular weight determination. Gas chromatography and mass spectroscopy combined to form GC-MS.

RESULTS & DISCUSSION

8. REULTS AND DISCUSSION

8.1. PHARMACOGNOSTICAL STUDIES:

Pharmacognostical studies play a key factor in establishing the authenticity of the plant material. The botanical identity of the seed was established by examining its anatomical features.

The results of the pharmacognostical studies are as follows.

Organoleptic characters

Nature - yellowish coarse powder

Colour - Yellowish orange

Odour - Aromatic

Taste - Bitter

Morphological Features:

Seed aril are oval or elliptical shaped and are 4-5cm in length and 2-3 cm in width. The thickness of the seed aril is mostly 0.5 to 1 cm. The seed are hard and brittle in nature. The seed aril is present in between endocarp and the sed.

The morphological features of the seed aril are tabulated below for a better and easy understanding.

Table No. 2: Morphological features of Seed aril

S.no	Parameters	Observation
1	Size:	4-5 cm in length
2	Shape:	Ovular
3	**Colour:**	Yellowish orange
4	**Odour:**	Aromatic
5	**Taste**	Bitter.

Fig. 13: Fresh fruit with seed aril

Fig. 14: Dried Seed Aril

MICROSCOPY:

The TS section are made by fixing the drug to the formalin and precisely sectioned used a microtome and stained. The characteristics of the T.S of seed are explained below

Transverse section of *Myristica malabarica seed aril*:

The seed aril are hard and brittle in nature. The transverse section of the seed aril showed the presence of the epidermal cell, parenchymal cells, oil glands and vascular bundle.

Epidermal cells:

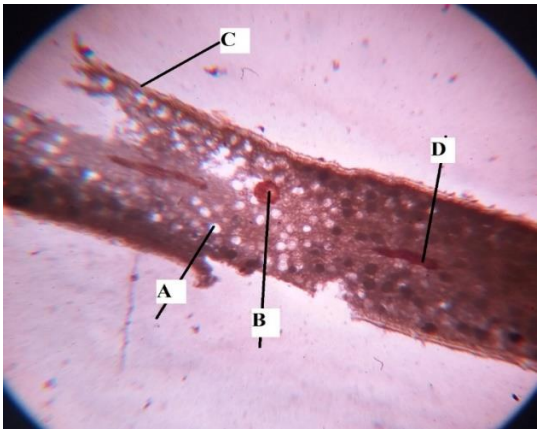
The epidermal cells are present at the outer layer. They are arranged without any intracellular spaces.

Parenchymatous cell:

Arranged horizontally along with the epidermal cells and oil glands and oil globules are arranged in these parenchymatous layer.

Vascular bundle:

Xylem and Phloem are arranged found in the seed aril. Crystal sheath and calcium oxalate are present in the parenchymatous layer of the seed aril.



A- Starch grains, B- Vascular bundle,C- Epidermal cells and D- Parenchyma cells.

Fig. 15: TS of *Myristica malabarica*

POWDER MICROSCOPY:

Powder microscopy of the seed aril of *Myristica malabarica* was observed and it showed the presence of fibers, xylem vessels, stone cells and calcium oxalate, oil cells and starch grains were also observed.

These features can be employed for inter specific identity of drugs.

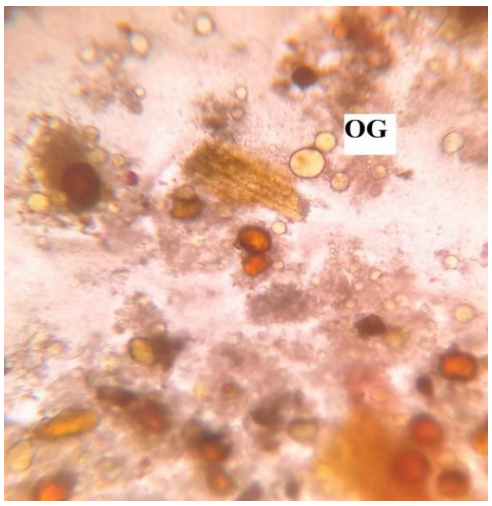


Fig. 16: Oil Globules

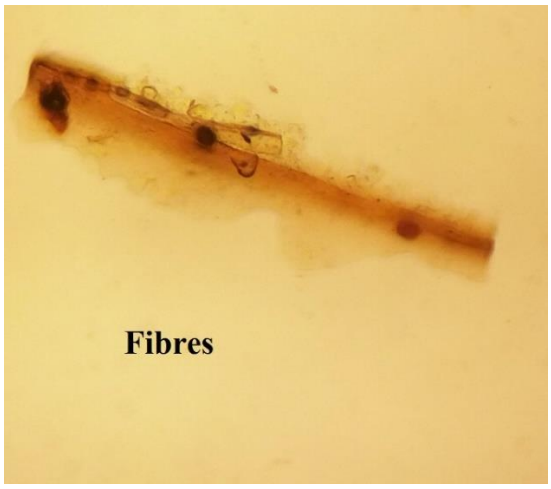


Fig. 17: Fibre

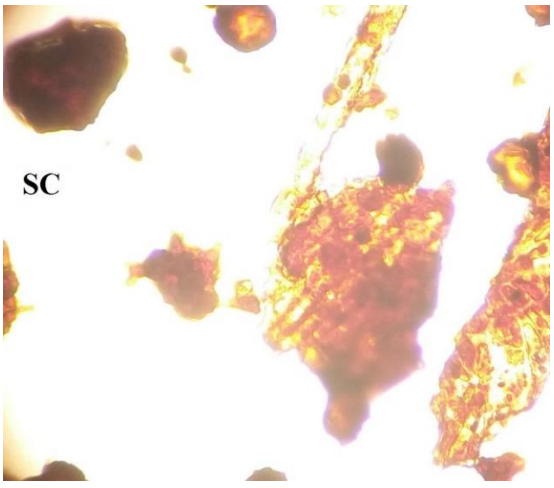


Fig. 18: Cluster of stone cell

Fig. 19 : Calcium oxalate crystal

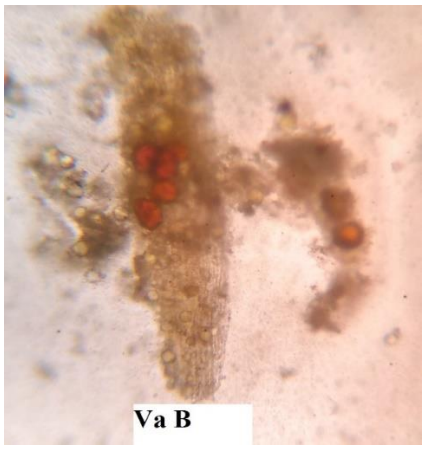


Fig. 20: Vascular bundle

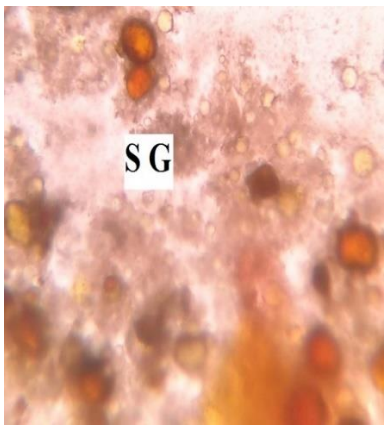


Fig. 21: Starch grains

HISTOCHEMICAL COLOUR REACTIONS:

Histochemical analysis is done in order to detect presence of phytoconstituents and their histology.

The Histochemical analysis showed the presence of Lignin, Starch and Tannin in xylem, Middle layer near oil globule and vascular bundle.

Table 3: Histochemical colour reactions of *Myristica malabarica.*

Sno	Chemicals	Test for	Nature of change	Histology	Degree of change
1	Phloroglucinol + HCl	Lignin	Pink	xylem	+
2	N / 50 Iodine Solution	Starch	Blue	Middle layer near	+
3	Dil. Ferric Chloride	Tannin	Black	Vascular bundles	+
4	Picric acid	Protein	No yellow colour	--	-
5	Dragendroff's reagent	Alkaloid	No orange colour	--	-

Note: + -- Indicates the prescence, - indicates absence

PHYSICO-CHEMICAL CONSTANTS

Physicochemical parameters are mainly used in judging the purity and quality of the powdered drug. Ash values of a drug give an idea of the earthy matter or inorganic elements and other impurities present along with the drug.

Ash values are mainly used in judging the purity and quality of the drug is the indicative of contamination, substitution and adulteration. The total ash usually consists of carbonate, phosphate and silicates. Total ash, acid insoluble ash and water soluble ash were done and reported in the table below.

Table No. 4: The Physicochemical analysis of the seed aril of *Myristica malabarica*.

S.NO	PHYSICO-CHEMICAL CONSTANTS	RESULTS (%w/w)
I	**ASH VALUE**	
1.	Total ash	3.26 ± 0.15
2.	Water soluble ash	0.72± 0.06
3.	Acid insoluble ash	0.89 ± 0.15
4.	Sulphated ash	1.00 ± 0.15
II	**EXTRACTIVE VALUE**	
1.	Water soluble extractive	28.34 ± 1.44
2.	Alcohol soluble extractive	10.56 ± 0.19
3.	Ether soluble extractive	2.22 ± 0.12
4	Nonvolatile ether soluble extractive	2.08± 0.14
III	**Loss on drying**	5.69 ± 0.19%
IV	**Swelling index**	1.5 ml
V	**Foaming index**	NIL
VI	**Volatile oil content**	6% V/W

Values are expressed as Mean ± SD, n=3

INORGANIC ELEMENTS AND HEAVY METAL ANALYSIS

Qualitative and quantitative estimation of inorganic metals were analyzed using Inductive coupled plasma analysis method and the results were tabulated as follows.

Table No. 5: Qualitative estimation of inorganic elements of *Myristica malabarica*.

S.No	INORGANIC ELEMENTS	OBSERVATIONS
1.	Aluminium	+
2.	Chloride	+
3.	Copper	-
4.	Calcium	+
5.	Iron	-
6.	Borate	+
7.	Potassium	+
8.	Carbonate	-
9.	Sulphates	-
10	Silver	-
11	Nitrate	+

Table No. 6: Quantitative estimation of inorganic elements of *Myristica malabarica*.

S.NO	INORGANIC ELEMENTS	TOTAL AMOUNT (% W/W)
1.	Aluminium	0.018
2.	Chloride	0.044
3	Calcium	0.032
4.	Borate	0.004
5.	Potassium	0.049
6.	Nitrate	0.011

Quantitative estimation of Heavy metals by ICP OES method

The quantification of the individual heavy metals was analyzed for the powdered mixture of *Myristica malabarica* by ICP-OES technique the following metals like arsenic, lead, cadmium were detected and quantified, results are given in the following table.

Table No. 7: Quantitative estimation of Heavy metals

S.No	Element	Results (ppm)	Specification
1.	Mercury	Not detected	Not more than 0.5ppm
2.	Arsenic	0.023	Not more than 5.0ppm
3.	Lead	0.032	Not more than 10ppm
4.	Cadmium	0.001	Not more than 0.3ppm

The above observation showed that the material is within the limits as per WHO standard and it is safe to consume internally.

8.2. PHYTOCHEMICAL STUDIES

EXTRACTION:

Extraction with the various solvent were performed, percentage yield and phytochemical analysis were done. The percentage yield of each extract are tabulated below.

Table No. 8: Percentage yield of successive extracts.

S.NO	EXTRACT	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD (%W/W)
1.	Benzene	Continuous Hot percolation method using Soxhlet apparatus	Semi solid	Orangish yellow	8.5
2.	Ethanolic		Semisolid and sticky	Brownish yellow	10.2
3.	Aqueous	Hydro distillation using Clevenger's apparatus	Liquid and sticky	Yellowish orange	6.5

PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of various extracts were performed and presence of flavonoid, tannins, saponins and steroids were significant.

Table No. 9: Qualitative Phytochemical analysis

SNO	TESTS	POWDER	BENZENE	ETHANOL	AQUEOUS
1	Alkaloids	-	-	-	-
2	Flavonoids	+	+	++	+
3	Phytosterols	+	-	+	-
4	Triterpenoid	+	-	+	+
5	Tannins and phenolic compound	+	-	+	+
6	Saponins	+	-	+	+
7	Gums and mucilage	+	-	+	+
8	Carbohydrates	+	-	+	+
9	Glycosides	-	-	-	-
10	Proteins & amino acids	-	-	-	-
11	Steroids	+	-	+	+
12	Fixed oils and Fats	-	-	-	-

Note: + indicates presence, - indicates absence

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

The *Myristica malabarica* Lam., was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them the estimation of total Flavonoids, Phenolic and Alkaloidal content in the ethanolic, aqueous and benzene extract are done.

TOTLA FLAVONOID CONTENT

Total flavonoid content was determined and compared with that of standard and thus ethanolic extract was found to have higher absorbance than benzene and aqueous extracts. It is represented in table

Table No. 10: Determination of Total Flavanoid content

S.No	Concentration (µg/ml)	Absorbance(765nm) of extracts			
		Standard	Benzene	Ethanolic	Aqueous
1.	20	0.07	0.04	0.05	0.04
2.	40	0.10	0.07	0.09	0.06
3.	60	0.13	0.10	0.12	0.09
4.	80	0.16	0.13	0.15	0.11
5.	100	0.20	0.15	0.18	0.14

Standard calibration curve for determination of Total Flavonoid Content

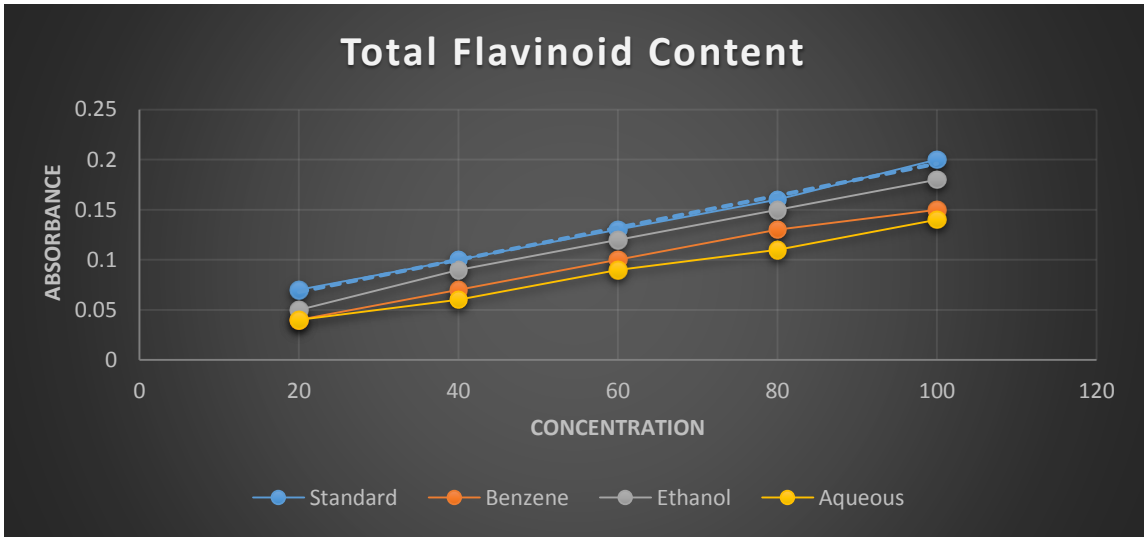


Fig. 22: Total Flavonoid content

T OTAL PHENOLIC CONTENT

Total phenolic content was determined and compared with that of standard and thus ethanolic extract was found to have higher absorbance than benzene and aqueous extracts. It is shown in Table

Table No. 11: Determination of Total phenolic content

S.No	Concentration (µg/ml)	Absorbance(765nm) of extracts			
		Standard	Benzene	Ethanolic	Aqueous
1.	20	0.13	0.09	0.11	0.04
2.	40	0.16	0.11	0.13	0.06
3.	60	0.19	0.13	0.16	0.09
4.	80	0.22	0.16	0.19	0.11
5.	100	0.25	0.18	0.22	0.14

StandardCalibration curve for determination of total Phenolic content

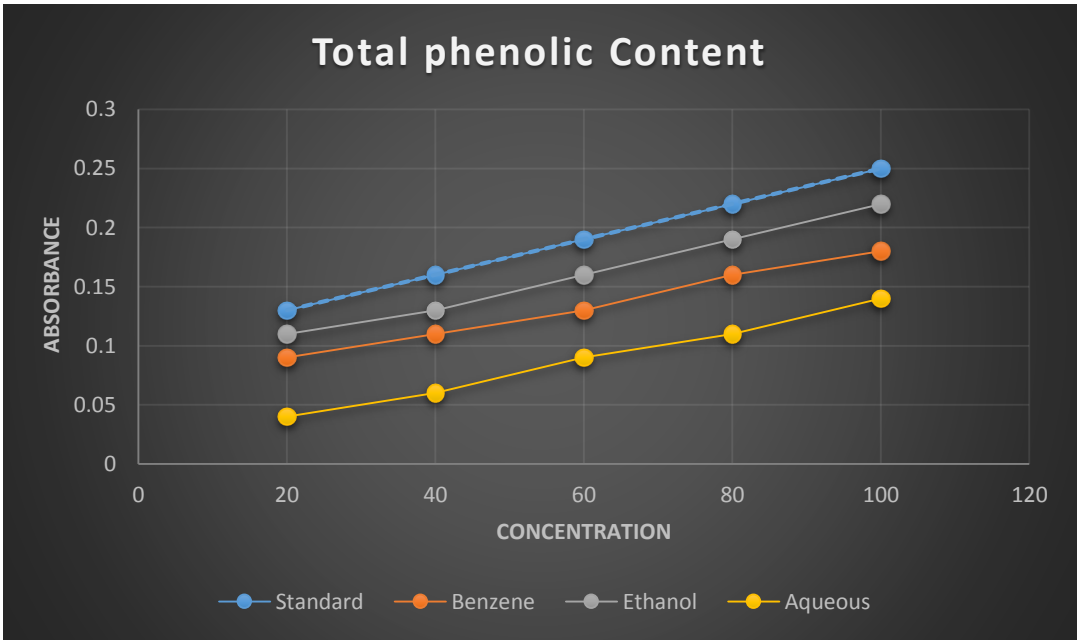


Fig. 23: Total Phenolic Content

Total Alkaloid content

Total alkaloid content was determined and compared with that of standard and thus ethanolic extract was found to have higher absorbance than benzene and aqueous extracts. It is shown in Table

Table No. 12: Total alkaloidal content

S.No	Concentration (µg/ml)	Absorbance(470nm) of extracts			
		Standard	Benzene	Ethanolic	Aqueous
1.	20	0.15	0.06	0.10	0.03
2.	40	0.17	0.08	0.12	0.05
3.	60	0.19	0.11	0.13	0.07
4.	80	0.21	0.14	0.15	0.13
5.	100	0.24	0.16	0.18	0.16

Standard Calibration curve for determination of total alkaloid content

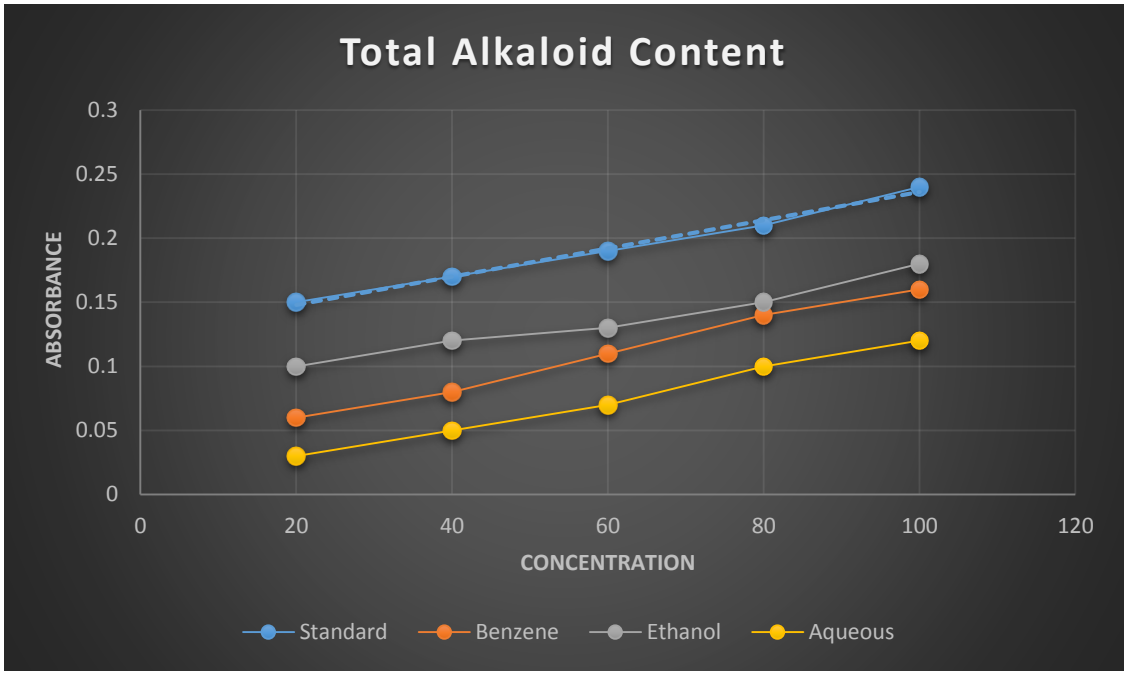


Fig. 24: Total Alkaloid Content

The Flavonoids, phenolic and alkaloids content present in the extract were determined quantitatively.

Total Flavonoid, phenol and alkaloid content of extracts of *Myristica malabarica* are represented in table.

Table No. 13: Determination of Total Phytochemical content

S.No	EXTRACT	TOTAL FLAVANOID CONTENT	TOTAL PHENOLIC CONTENT	TOTAL ALKALOID CONTENT
1.	Benzene	50.25 µg/mg	5.250µg/mg	3.45 µg/mg
2.	Ethanol	70.42 µg/mg	8.375µg/mg	5.31 µg/mg
3	Aqueous	42.51 µg/mg	3.81 µg/mg	2.15 µg/mg

FLUORESCENCE ANALYSIS

Fluorescent analysis of powder and extracts were analyzed in day light and under UV of 265 nm and 365 nm.

Table No. 14: Fluorescence characteristic of powdered samples at short UV and long UV.

TREATMENT	DAY LIGHT	SHORT UV (254 nm)	LONG UV (365 nm)
Drug	Pale white	Dark brown	Orange
Drug + Water	Light yellow	Light yellow	Orange
Drug + 1 N Hcl	Light yellow	Dark green	Pale yellow
Drug + 1 N HNO_3	Light yellow	Dark green	Brownish yellow
Drug + 1 N H_2SO_4	Reddish brown	Blackish brown	Dark brown
Drug + Acetic acid	Light yellow	Pale green	Yellowish brown
Drug + 1N NaOH	Dark yellow	Greenish brown	Orange
Drug + 1N NaOH	Dark yellow	Greenish brown	Orange
Drug + 1 N KOH	Dark yellow	Greenish brown	Pale brown

Drug + 1N KOH	Dark yellow	Greenish brown	Pale brown
Drug + Ammonia	Dark yellow	Dark green	Dark orange
Drug + Iodine	Brownish orange	Brownish green	Reddish green
Drug + $Fecl_3$	Brownish green	Brownish green	Dark green
Drug + Picric acid	Dark yellow	Dark green	Brownish green

Florescence characteristics of the extracts of the seed aril of *Myristica malabarica.*

Table No. 15: Fluorescence characteristic of extracts at short UV and long UV.

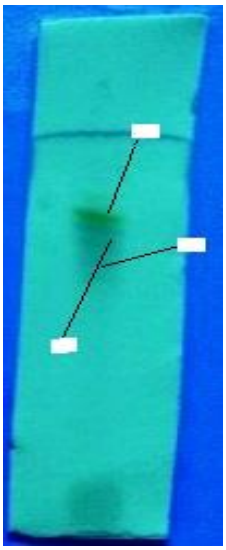
EXTRACT	**DAY LIGHT**	**SHORT UV (254 nm)**	**LONG UV (365 nm)**
Hexane	Pale brown	Reddish brown	Brown
Aqueous	Brownish black	Blackish green	Dark Brown
Ethanolic	Brown	Reddish brown	Brown

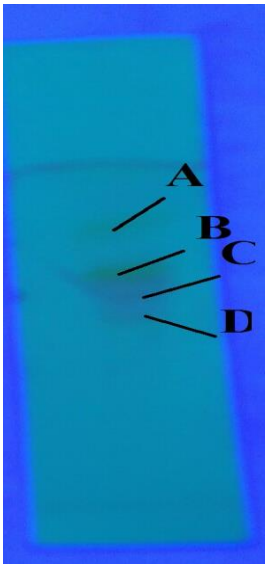
TLC OF EXTRACTS:

Thin Layer Chromatogrphy of the various extracts were performed and R_f value was determined.

Table No. 16: Thin layer chromatography of various extract.

SNO	**EXTRACTS**	**SOLVENT SYSTEMS**	**NO OF SPOTS**	**R_f value**
1	Benzene	Methanol : Chloroform 1:9	3	0.86 0.81 0.75
2	Ethanolic	Methanol : Chloroform 1:9	4	0.88 0.85 0.83 0.81
3	Aqueous	Methanol : Chloroform 1:9	2	0.74 0.68





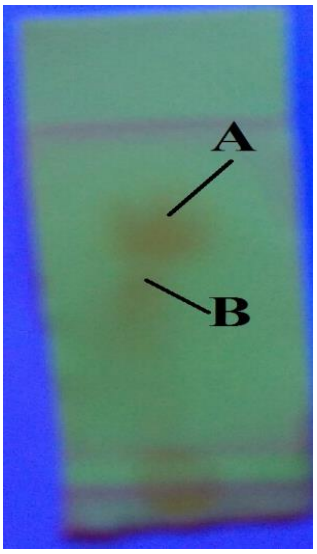


Fig. 25: Benzene TLC **Fig. 26: Ethanoli TLC** **Fig. 27: Aqueous TLC**

TLC of all the three extracts were run through various solvents. An effective separation was found in the Ethanol: Ethyl acetate: Water solvent system. Separation was incomplete when solvents for alkaloid, steroids and tannins were performed. It was found to contain flavonoids.

HPTLC FINGER PRINT PROFILE

HPTLC Finger print Data of Ethanolic Extract of *Myristica malabarica* Lam.,

High performance thin layer chromatography (HPTLC) finger printing was performed with the ethanol extract of ***Myristica malabarica* Lam.,** The chromatographic conditions were carried as detailed in material and method of this study. There were 10 peaks observed with different Rf Values and different heights.

Table No. 17: Solvent system of HPTLC analysis

EXTRACT	**SOLVENT**
Ethanolic extract	Hexane : Ethyl acetate : Chloroform : Toulene : Methanol : Formic acid (2: 2 : 1: 4: 0.5: 0.5) Derivatizing agent: 10% Anisaldehyde sulphuric acid Lamp: Tungsten

Fig. 28: HPTLC Finger Print Data

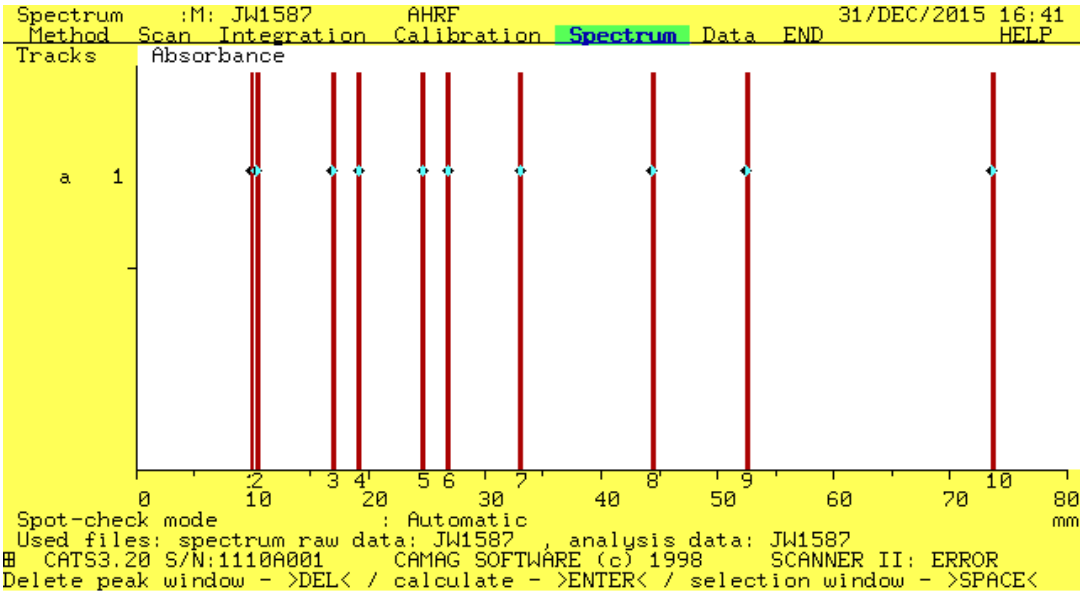


Fig. 29: HPTLC chromatogram for *Myristica malabarica* Lam

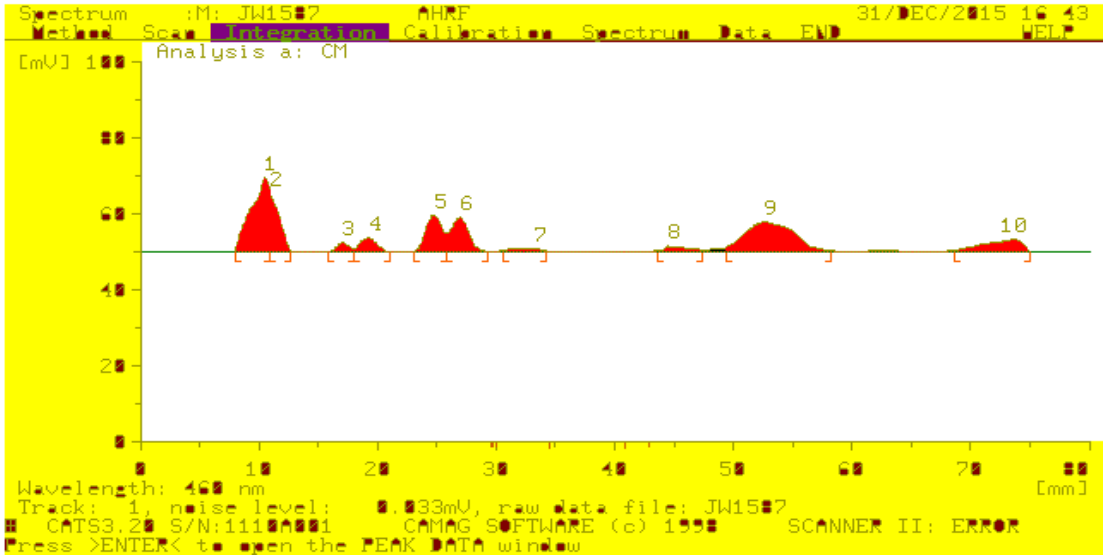


Fig. 30: Histogram for *Myristica malabarica* Lam HPTLC data

Table No. 18: HPTLC profile of extracts

S.No	Rf	Heigh	Area	Lamda Max
1	0.10	19.6	342.8	737
2	0.11	15.2	131.4	800
3	0.17	2.4	28.6	507
4	0.19	3.6	88.3	507
5	0.25	9.7	156.7	507
6	0.27	9.0	153.5	507
7	0.33	0.9	25.0	507
8	0.44	1.5	34.5	507
9	0.52	7.8	382.1	507
10	0.74	3.3	124.7	507

8.3 PHARMACOLOGICAL STUDIES

8.3.1 ANTIOXIDANT ACTIVITIES

A number of studies indicates that oxidative stress is also a factor for sleep disorder which disrupts the GABA receptor binding activity which is essential for sleep. GABA binding proteins are inhibited by free radicals. Likewise, there are numerous studies which have examined the positive benefits of antioxidants linked to sleep disorder. In addition, the antioxidant potential of a compound can be attributed to its radical scavenging ability and total antioxidant activity. In order to determine the ability of the plant extracts to serve as antioxidants, two activities were measured; ability to scavenge DPPH and Hydrogen Peroxide Scavenging assay.

DPPH radical scavenging activity:

Various extracts were studied for its anti-oxidant activity using 1, 2-diphenyl-2-picryl hydrazyl radical (DPPH). Results are discussed below.

Table No. 19: DPPH Radical Scavenging activity of various extract

S.No	Concentration (µg/ml)	% Inhibition			
		Standard (Butylated hydroxyl Toulene)	Benzene extracts	Ethanolic extracts	Aqueous extracts
1.	20	54.16	34.43	48.94	21.34
2.	40	63.51	46.32	55.86	31.17
3.	60	77.11	51.54	69.18	38.36
4.	80	80.95	62.34	75.95	47.32
5	100	93.36	73.34	89.87	52.45
IC_{50} (µg/ml)		10	36	24	61

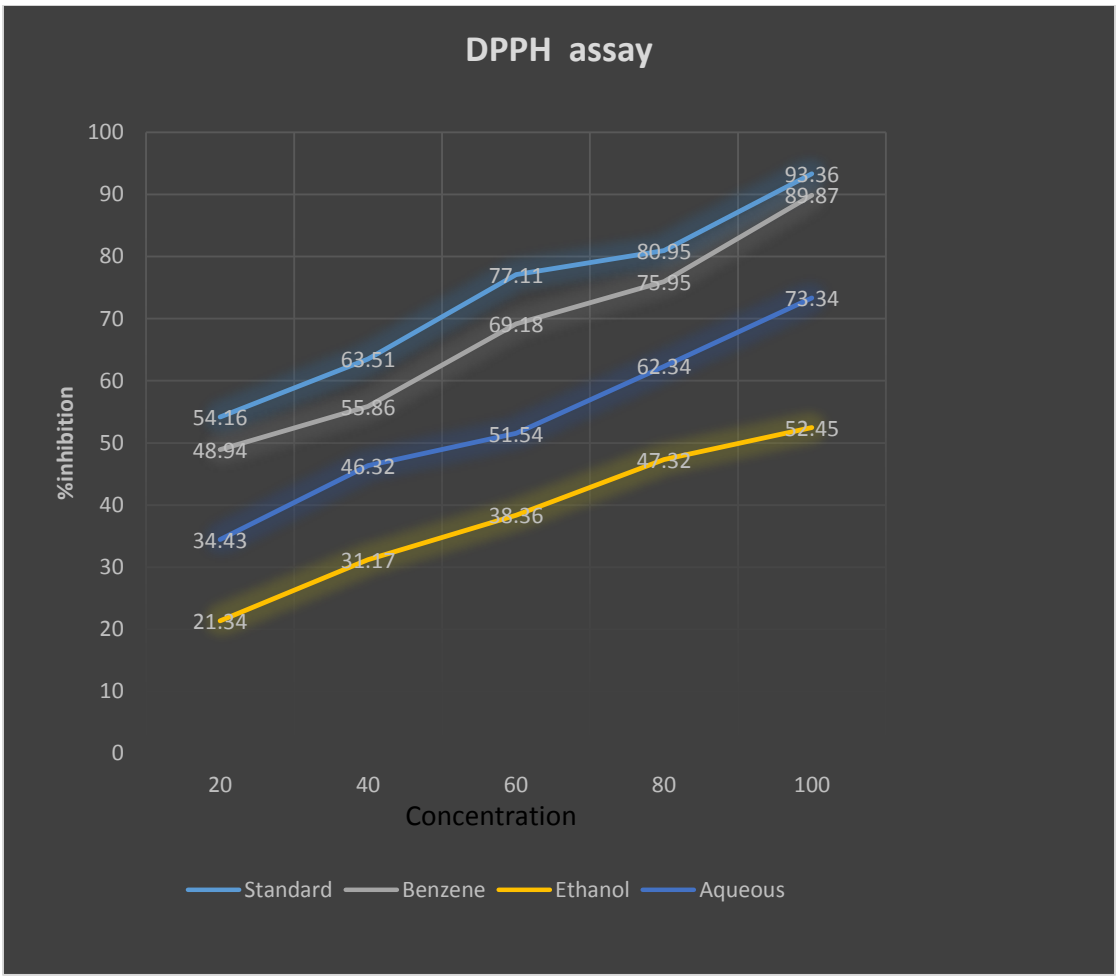


Fig. 31: DPPH Radical Scavenging activity of various extract

The DPPH is a stable free radical which produced deep purple colour by accepting the proton from any proton donor substance and widely used to test free radical scavenging effect. The reduction of DPPH decreases its absorbance at 517 nm due to colour change. The ethanolic extract of *Myristica malabarica* showed IC_{50} value (µg/ml) of 24 whereas Benzene and aqueous extract showed Ic_{50} (µg/ml) value 36 and 61 respectively. Whereas the IC_{50} (µg/ml) value of standard BHT was 10. Ethanolic extracts of *Myristica malabarica* is found to produce good scavenging activity in comparison to standard.

HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY:

The benzene, ethanol and aqueous extracts were subjected to hydrogen peroxide radical scavenging assay and the results are tabulated below in Table 2 & Fig 2.

Table No. 20: Hydrogen peroxide Radical Scavenging activity of various extract

Sno	Concentration	% inhibition			
		Standard (Ascorbic acid)	Aqueous extracts	Benzene extracts	Ethanolic extracts
1	20 µg/ml	35.14	12.34	21.71	32.43
2	40 µg/ml	42.34	24.54	34.23	41.65
3	60 µg/ml	58.84	28.45	41.43	51.81
4	80 µg/ml	75.32	32.54	49.34	59.65
5	100 µg/ml	89.74	38.56	54.43	63.56
IC_{50} (µg/ml)		37	127	68	54

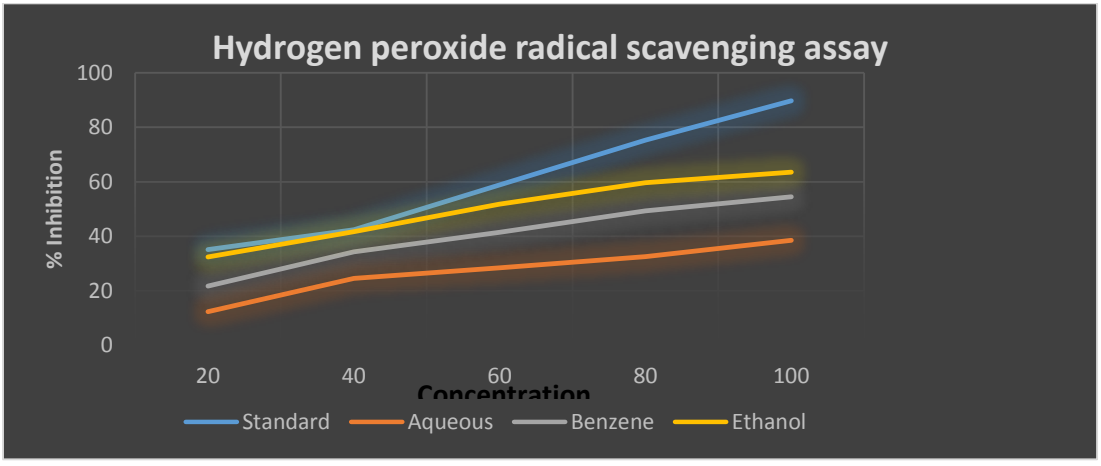


Fig. 32: Hydrogen peroxide Radical Scavenging activity of various extract

The ability of the extract to scavenge hydrogen peroxide was determined in this method. Absorbance is measured at 230 nm and IC_{50} (µg/ml) value has been calculated and was found to be 54 for Ethanolic extract, whereas Benzene and Aqueous extract showed the IC_{50} –value (µg/ml) of 68 and 127 respectively. IC_{50} value of standard Ascorbic acid was 37.

Ethanolic extract of *Myristica malabarica* was found to produce good scavenging activity in comparison to standard.

The below bar diagram repesents the IC_{50} value for different extracts using DPPH and Hydrogen peroxide scavenging method. The lower the IC_{50}value the higher the antioxidant activity. From the result Ethanolic extract has the lowest value, Benzene has moderate value and aqueous has highest IC_{50} value. Therefore it is evident that Ethanolic extract has high antioxidant property where Aqueous extract has the lowest antioxidant property.

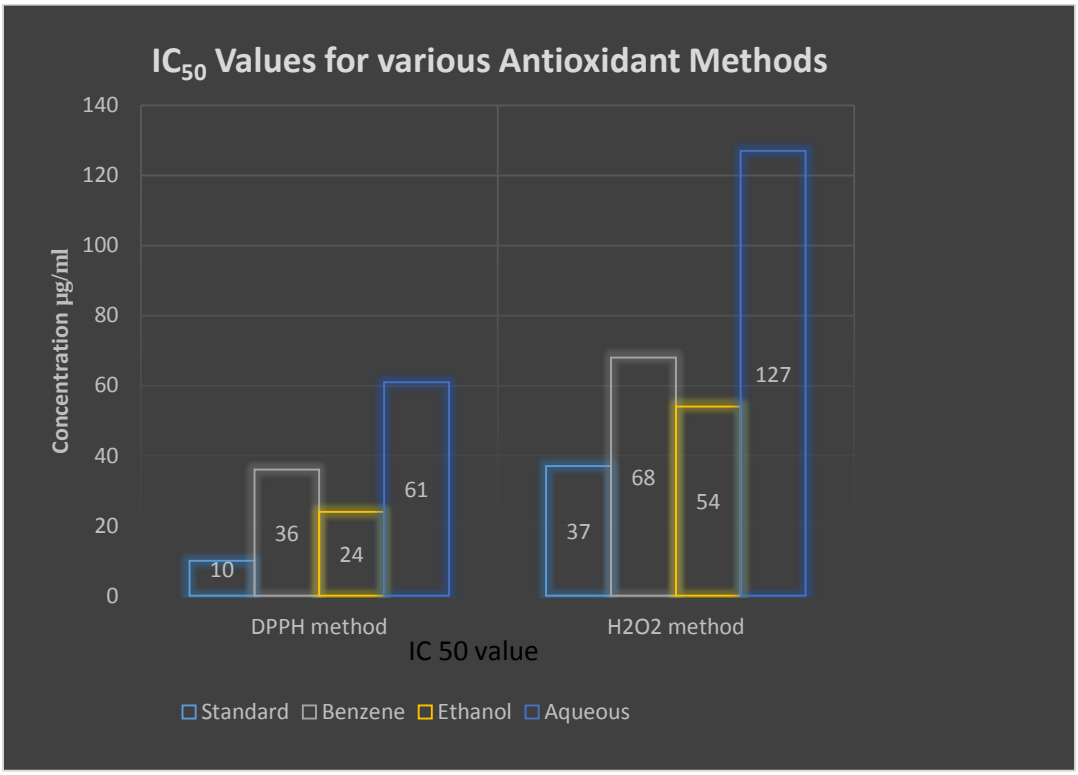


Fig. 33: IC_{50} values of antioxidant activity of various extract

8.3.2 IN-VIVO STDIES

EVALUVATION OF SEDATIVE ACTIVITY BY SPONTANEOUS LOCOMOTOR ACTIVITY

The three extracts of *Myristica malabarica* (Benzene, Ethanolic and Aqueous) are subjected for Sedative activity by Actophotometer method. The number of crossing by the mice before and after the drug administration is noted and the results are listed below.

Table No. 21: Sedative activity using actophotometer

S.No	Group	No of Crossings Before and after drug adminstration		
		Before	**30mins after**	**60mins after**
1	Control (1% tween 80)	252.43±8.21	249.69±7.32	251.62±2.10
2	Standard (Diazepam)	241.21±6.15	86.53±5.74	104.30±4.91
3	Benzene extract (400mg/kg)	239.56±4.54	107.82±4.09	113.41±5.72
4	Ethanolic extract (400mg/kg)	250.08±3.11	92.29±5.39	108.71±2.60
5	Aqueous extract (400mg/kg)	247.11±5.56	121.19±6.17	134.27±7.61

Mean ±SEM; *n=6 in each group;* One way ANOVA

Table No. 22: Percentage decrease in Locomotor activity

S.No	Group	% Decrease in locomotor activity compared to standard
1	Control (1% tween 80)	36.21
2	Standard (Diazepam)	100
3	Benzene extract (400mg/kg)	86.36
4	Ethanolic extract (400mg/kg)	95.01
5	Aqueous extract (400mg/kg)	74.83

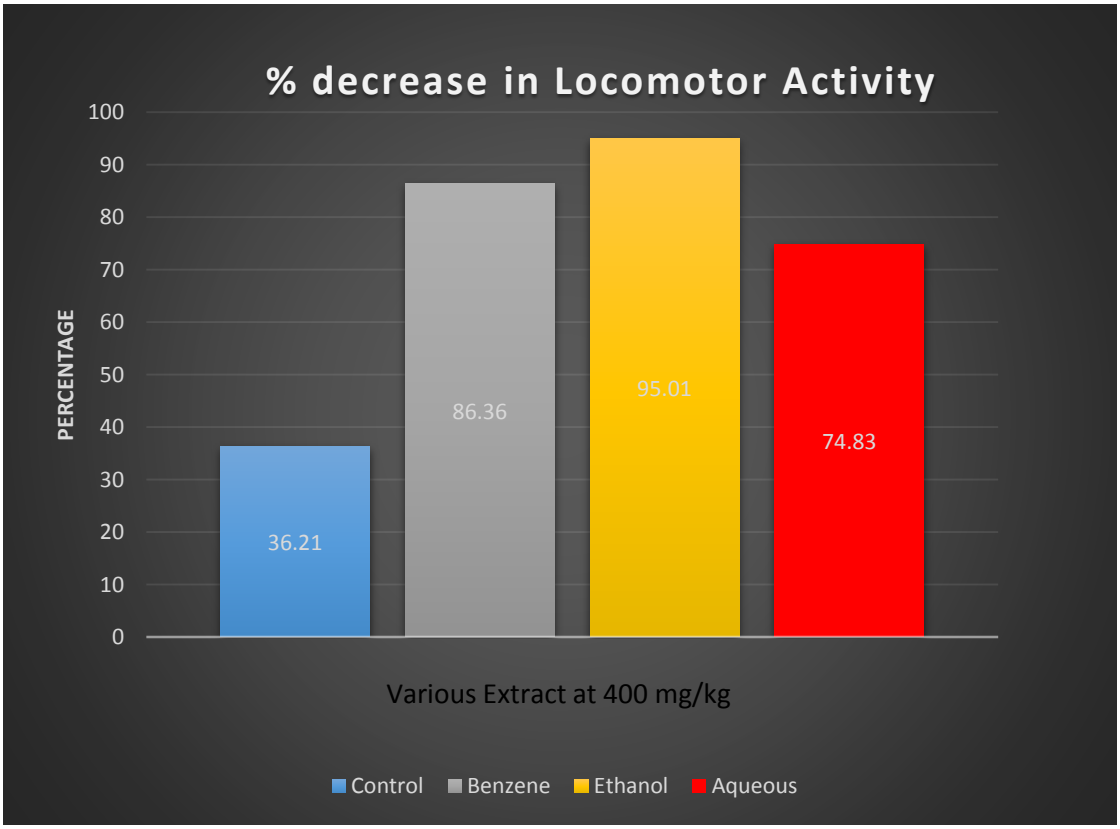


Fig. 34: Percentage decrease in locomotor activty

The three extracts was subjected to sedative activity which is determined by spontaneous locomotor activity in mice and the results show that all three extracts has significant sedative activity.

The Ethanolic extract has the highest activity of 95.01%, Benzene extract has moderate activity of 86.36 % and the least active is aqueous extract has 74.83% sedative activity when compared to the standard drug Diazepam. Ethanolic extract has the nearest value to the standard hence it has the highest hypnotic activity among extracts

All values are expressed as Mean ±SD and datas were analysed by One Way Annova followed by Dunnett's test a-P<0.05: b-P<0.01 when compared with standard group.

EVALUVATION OF HYPNOTIC ACTIVITY BY POTENTIATION OF HEXOBARBITAL INDUCED SLEEP TIME

The three extracts of *Myristica malabarica* (Benzene, Ethanolic and Aqueous) are screened for hypnotic activity. The standard drug and extracts were given 30 mins before the administration of Hexobarbital. The onset of sleep and waking is determined by righting reflex of the mice used. The results are noted and listed below in tabular column and in graphical bar representation for a better understanding.

Table No. 23: Potentiation of Hexobarbital induced sloe time for Hypnotic activity

S.no	Group	Onset of sleep (min)	Duration of sleep (min)	% Increase in sleep compared to control
1	Control	5.25±6.28	95.57±7.21	-
2	Standard	3.19±7.11	124.32±8.22	31.57
3	Benzene (400 mg/kg)	4.14±11.45	102.41±6.42	7.36
4	Ethanolic (400 mg/kg)	3.52±5.24	118.27±9.51	24.21
5	Aqueous (400 mg/kg)	4.49±4.51	98.11±4.56	3.15

Mean ±SEM; *n=6 in each group;* One way ANOVA

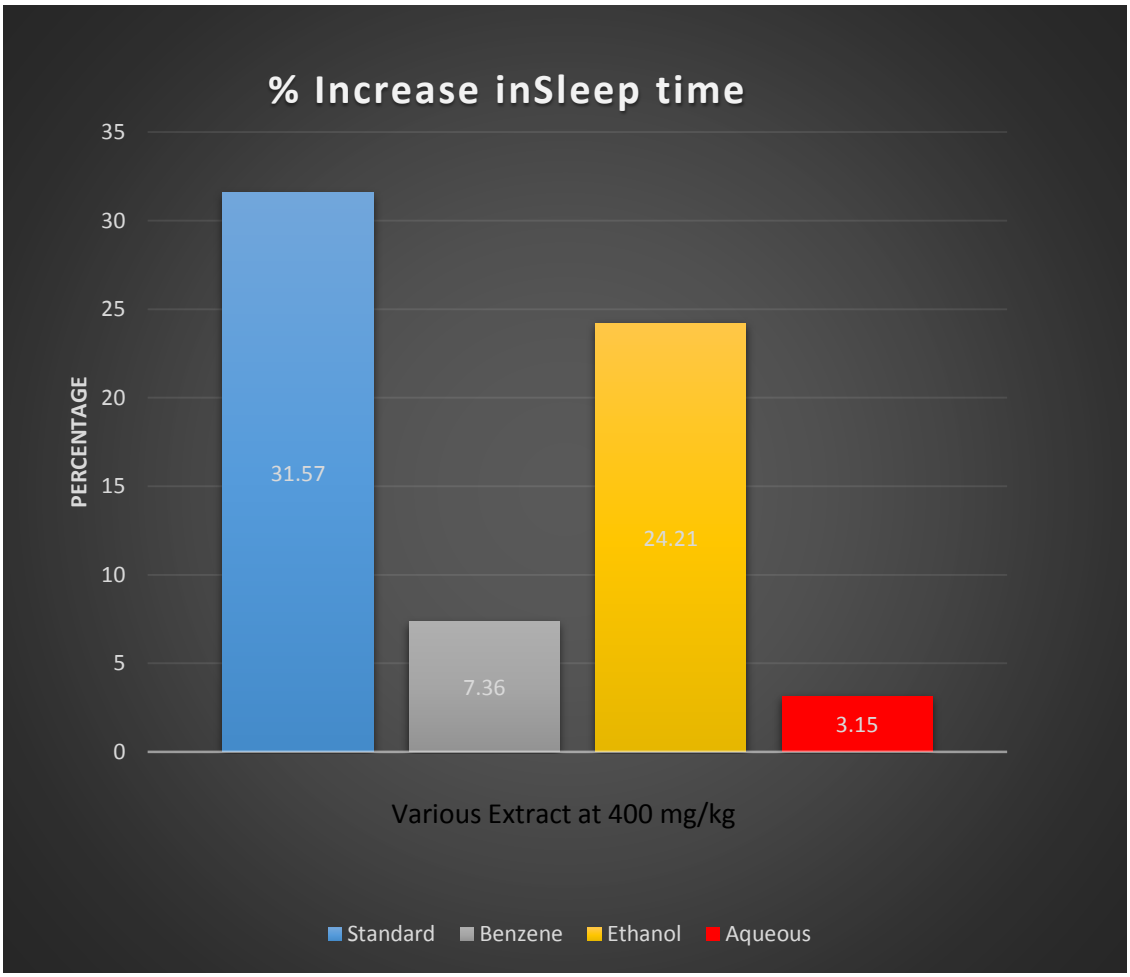


Fig. 35: Percentage increase in sleep time.

All three extracts has significant Hypnotic activity. The Ethanolic extract has the highest activity of 24.21%, Benzene extract has moderate activity of 7.36 % and the least active is aqueous extract has 3.15% hypnotic activity when compared to the standard drug Diazepam which has 31.57% potentiation of hexobarbital induced sleep time. Ethanolic extract has the nearest value to the standard hence it has the highest hypnotic activity among extracts.

All values are expressed as Mean ±SD and datas were analysed by One Way Annova followed by Dunnett's test a-P<0.05: b-P<0.01 when compared with standard group.

ISOLATION OF COMPOUND BY COLUMN CHROMATOGRAPHY:

A).COLUMN CHROMATOGRAPHY

The eluates obtained from silica gel column chromatography of ethanolic extract with different fraction are tabulated in table

Table No. 24: Fractionation of ethanolic extract

ELUENT	SOLVENT RATIO	FRACTION
n-Hexane	100%	0
Ethyl acetate: Hexane	10:90	1-3
Ethyl acetate: Hexane	20:80	4-6
Ethyl acetate: Hexane	30:70	7-11
Ethyl acetate: Hexane	40:60	12-16
Ethyl acetate: Hexane	50:50	17-21
Ethyl acetate: Hexane	60:40	22-26
Ethyl acetate: Hexane	70:30	27-31
Ethyl acetate: Hexane	80:20	32-36
Ethyl acetate: Hexane	90:10	37-41
Ethyl acetate	100%	42-46
Ethyl acetate: Ethanol	90:10	47-50

Ethyl acetate: Ethanol	80:20	51-54
Ethyl acetate: Ethanol	70:30	55-58
Ethyl acetate: Ethanol	60:40	59-62
Ethyl acetate: Ethanol	50:50	63-66
Ethyl acetate: Ethanol	40:60	67-70
Ethyl acetate: Ethanol	30:70	71-74
Ethyl acetate: Ethanol	20:80	75-78
Ethyl acetate: Ethanol	10:90	79-82
Ethanol	100%	83-86

Thin layer chromatography of isolated fractions

Solvent system:

Methanol: Chloroform (1:9)

Table No. 25: Thin layer chromatography of isolated fractions

SNO	FRACTION	COMPOUND	Rf VALUE
1	0	-	-
2	1-3	-	-
3	4-6	-	-
4	7-11	-	-
5	12-16	-	-

6	17-21	-	**-**
7	22-26	-	**-**
8	27-31	-	**-**
9	32-36	-	**-**
10	37-41	-	**-**
11	42-46	-	**-**
12	47-50	C1	**0.7**
13	51-54	C1	**0.7**
14	55-58	C1	**0.7**
15	59-62	C1	**0.7**
16	63-66	C1	**0.7**
17	67-70	C2	**0.8**
18	71-74	C2& 3	**0.8** **0.85**
19	75-78	C2&3	**0.8** **0.85**
20	79-82	C2&3	**0.8** **0.85**
21	83-86	C2&3	**0.8** **0.85**

Characterization of isolated compound

Physical properties of the isolated compounds are evaluated, such as

- Colour : Orangish yellow
- Nature : Solid
- Solubility : Soluble in Ethanol and Chloform
- Molecular weight : 358.434.
- Molecular formula : $C_{21}H_{26}O_5$
- Boiling point :263.434^{0}C

TLC of Compound 1

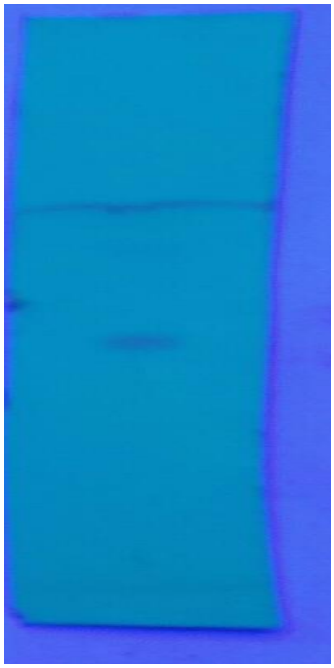


Fig. 36: TLC of Compound 1

Mobile phase: Methanol - Chloroform(1:9)

Stationary Phase: Silica gel G

No of spots: 1

Rf value: 0.8

Spectral analysis:

The compound C1 obtained from ethanolic extract have identified and spectral datas were depicted in below informations.

IR analysis

- IRυ^{cm-1}: KBr Pellet method.

Table No. 26: IR Interpretation

WAVE NUMBER	INTENSITY	TYPE
3390	Broad	O-H stretching
2926	Sharp	Benzene
2090	Weak band	C-H stretching in CH_2 and CH_3
2856	Sharp	C-H Stretching in CH_3
1900-1600	Moderate	>C= O Carbonyl group
1650-1450	Sharp	Aromatic C=C stretching
1451	Sharp	C-H stretching
1382	Sharp	C-H def Methyl
1272	Moderate	Phenol
1077	Moderate	C-O-C stretching
775	Sharp	Ortho substituted benzene
970-700	Sharp	$-CH=CH_2$

IR Spectral Analysis for compound C1

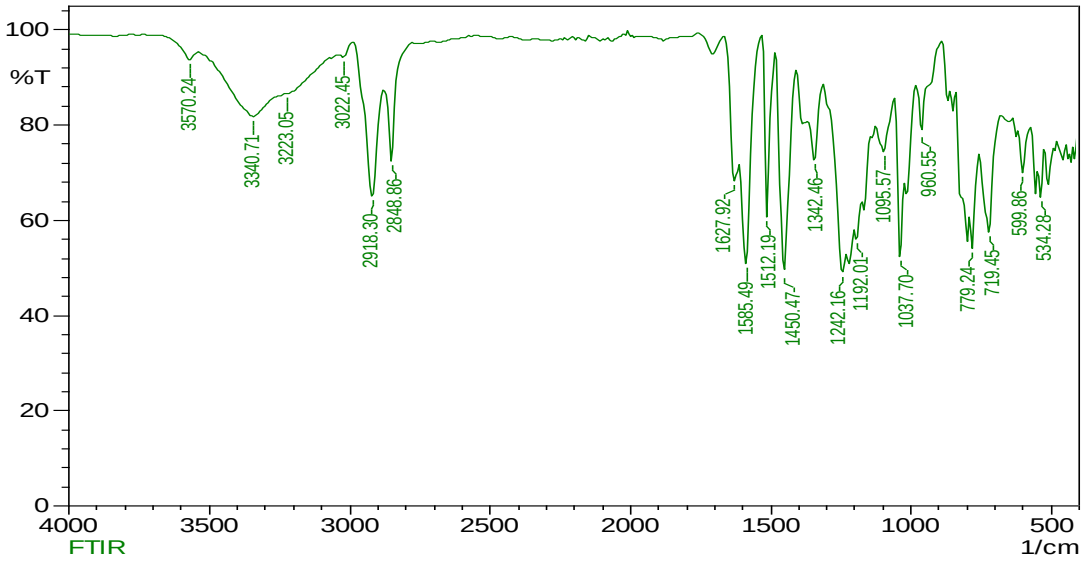


Fig. 37: IR analysis of Compound 1

FTIR Spectral Analysis for compound C1

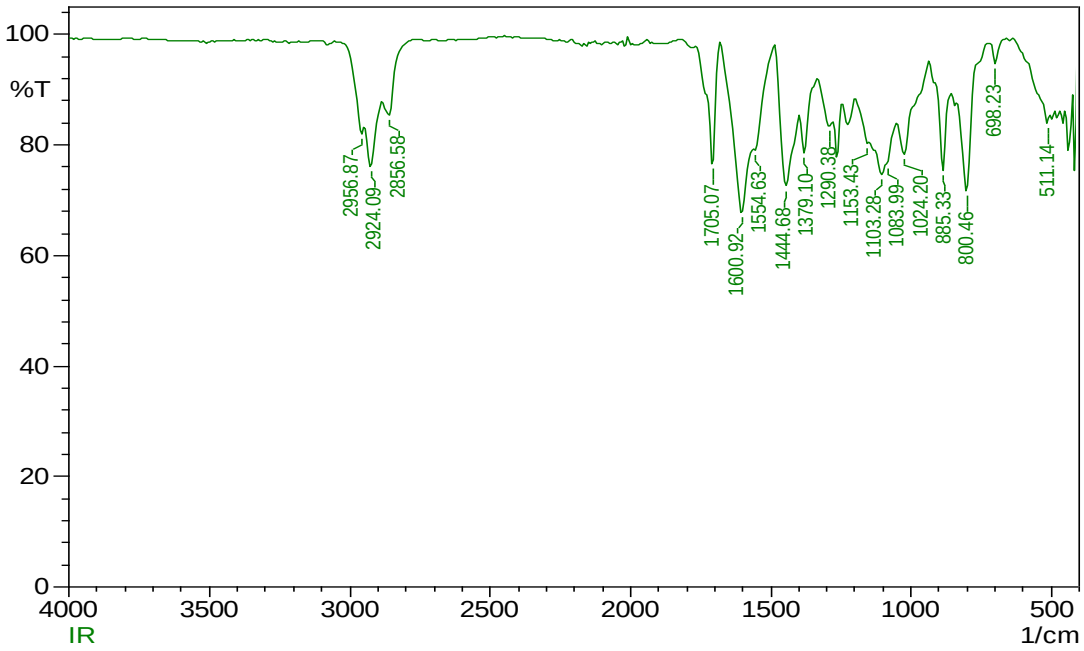


Fig. 38: FTIR analysis of Compound 1

GCMS analysis.

The GCMS analysis revealed the molecular structure through the following datas

Table No. 27: GCMS analysis of Compound 1

#	RT	Scan	Height	Area	Area %	Norm %
1	16.579	2755	68,070,480	40,475,748.0	16.009	32.44
2	17.179	2875	50,544,136	24,992,804.0	9.885	20.03
3	21.441	3727	35,927,156	7,395,712.0	2.925	5.93
4	23.326	4104	861,835,968	124,767,248.0	49.347	100.00
5	23.662	4171	83,498,344	9,893,860.0	3.913	7.93
6	25.587	4556	53,232,544	12,720,513.0	5.031	10.20
7	26.668	4772	34,031,532	9,056,013.0	3.582	7.26
8	27.038	4846	41,355,216	8,806,730.0	3.483	7.06
9	27.748	4988	37,612,764	14,727,990.0	5.825	11.80

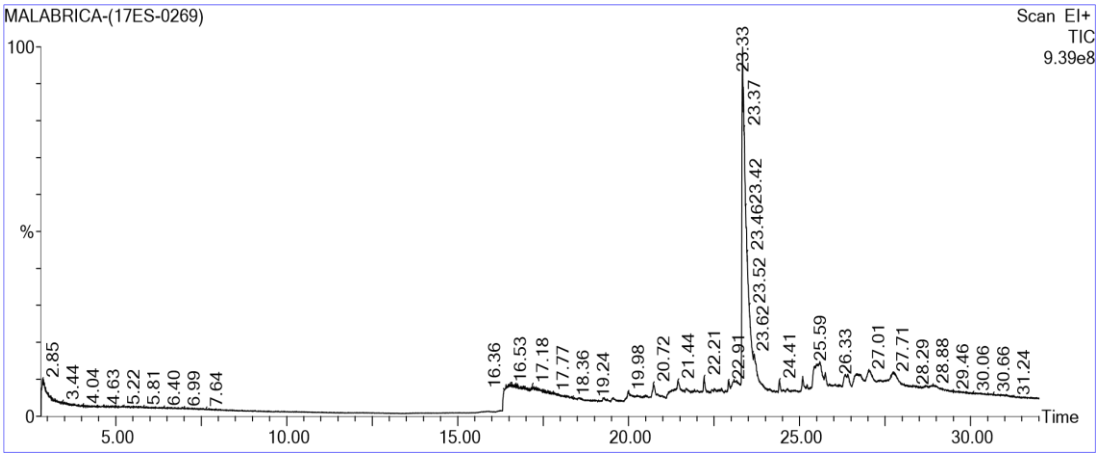


Fig. 39: GCMS Analysis of Compound 1

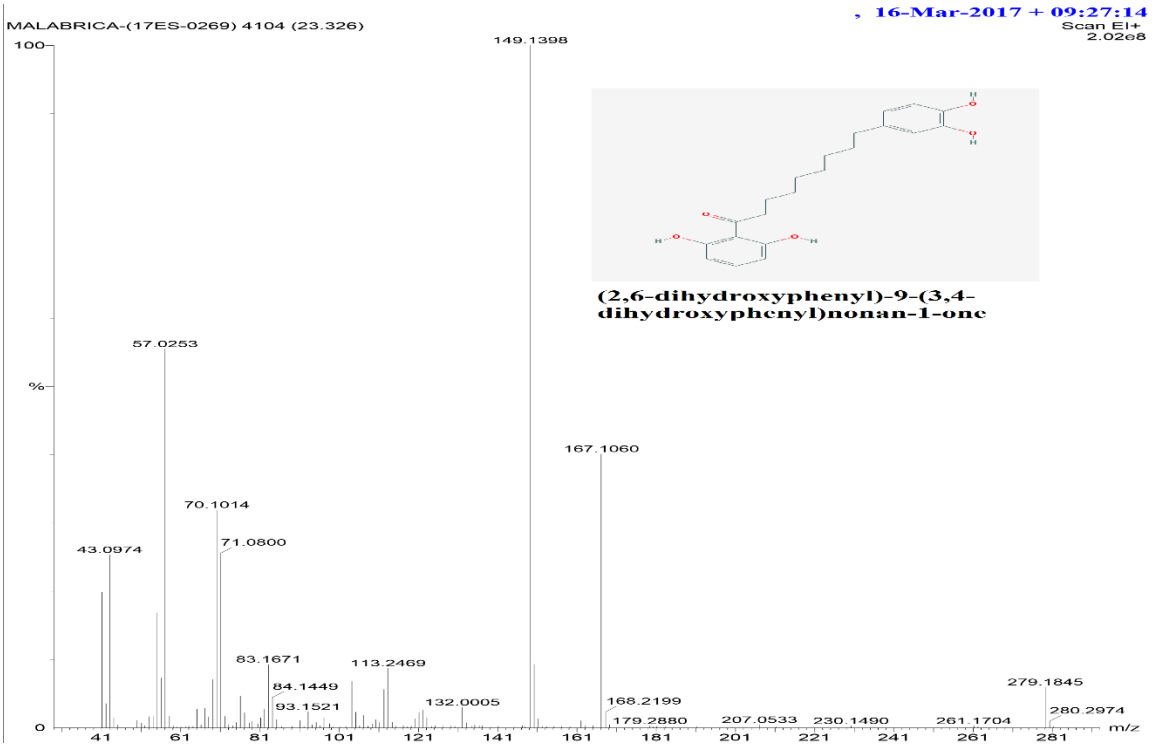


Fig. 40: GCMS Analysis of Compound 1 with structure

From the data obtained through IR, FTIR and GCMS analysis the isolated compound can be assumed to be Malabaricone C with IUPAC name as (2,6-dihydroxyphenyl)-9-(3,4-dihydroxyphenyl)nonan-1-one.

SUMMARY & CONCLUSION

9. SUMMARY AND CONCLUSION

Modern lifestyle has a great impact on people health and Sleep disorder is one of the most common disease that affects 1/5 of the population in India. Most of the drugs that is currently prescribed has undesirable side effects. Herbal medicines have greater potential in therapeutic medicine and has more patient compatibility with less desirable side effects. Herbal medicine is a major component in all indigenous traditional medicine and a common element in Ayurveda, Siddha, Homeopathy and Naturopathy systems of medicine. Hence the present study is an attempt to evaluate sedative and hypnotic activity on the seed aril of the plant *Myristica malabarica* Lam.,

9.1 PHARMACOGNOSTICAL STUDIES

- The pharmacognostical studies on the seed aril of *Myristica malabarica* Lam., was carried out, which showed the unique features of the bark which is used to differentiate it from other species.
- **Macroscopical studies** states the characteristic features of seed aril such as colour, shape, length and width, nature of the drug, smell, taste and other morphological characters has been well studied.
- **Microscopical analysis** of the seed aril along with TS showed the presence of oil globules, fibers, sclerides, calcium oxalate crystals, starch grains, etc, which can be as a standard for identification.
- **Physicochemical studies** various physiochemical constants were evaluated such as ash values, extractive values, volatile oil content, foreign organic matter, foaming index, swelling index and loss on drying.

These pharmacognostical parameters evaluated are useful for the establishment of standards of seed aril which is essential for its future references such as identity and purity of the drug.

9.2 PHYTOCHEMICAL STUDIES

- **In phytochemical study**, the powdered drug was successively extracted with benzene and ethanol by using soxhlet apparatus. Aqueous extraction is done by Clevenger's distillation apparatus. The percentage yield for benzene, ethanolic and aqueous extracts are 8.5, 10.2 and 6.5 % w/w.
- **Preliminary phytochemical investigation** was done for the powdered drug and all the extracts. It was found to contains flavonoids, triterpenoids, alkaloids, phenolic compounds, steroids, etc,.
- **Quantitative estimation** of the phytoconstituents is carried out for alkaloids, phenolic compounds and flavonoids.
- **Fluorescence analysis** was done to find out characteristic fluorescent substance present in the powdered bark and all the extracts, and no fluorescent substance was found.
- **High performance thin layer chromatography (HPTLC)** was performed with ethanolic extract of *Myristica malabarica* Lam., and the finger print showed the presence of 10 peaks helpful in the quantitative and qualitative identification of phytoconstituents.

9.3 PHARMACOLOGICAL ACTIVITY

***In vitro* studies**

- All the extracts were subjected to *in-vitro* antioxidant activity using DPPH and hydrogen peroxide scavenging method. All the extracts showed significant antioxidant activity and the ethanolic extract showed the maximum antioxidant potential. Therefore all the extracts were chosen for the *in-vivo* studies.

***In vivo* studies**

- *In-vivo* sedative and hypnotic activity was carried out for all the extract in Swiss albino mice by using spontaneous locomotor activity and potentiation of hexobarbital induced sleep time. The dose of the extracts is fixed to 400mg/kg through oral route.

- *In-vivo* sedative activity is evaluated by spontaneous locomotor activity using actophotometer. All the extracts showed sedative activity in which ethanolic extract has the highest activity. The percentage decrease in locomotor activity of the Ethanolic, Benzene and Aqueous extracts are 95.01, 86.36 and 74.83 when compared to the standard drug diazepam.

- *In-vivo* hypnotic study is evaluated by potentiation of hexobarbital induced sleep time. All the extracts showed hypnotic activity in which ethanolic extract has the highest activity. The percentage increase in sleep time of the Ethanolic, Benzene and Aqueous extracts are 24.21, 7.36 and 3.15 when compared to the control drug hexobarbital.

9.4 ISOLATION AND IDENTIFICATION

- The active constituents of the ethanolic extracts are isolated using column chromatography and spectral studies were done on the isolated compound.

- The IR, FTIR and GCMS spectral analysis data the compound was useful in identification of the compound as Malabaricone C with IUPAC name as (2,6-dihydroxyphenyl)-9-(3,4-dihydroxyphenyl)nonan-1-one.

From the above mentioned reports it can be concluded that the standards generated will be useful in proper identification of plant and also to differentiate it from its closely related species and adulterants with the support of Pharmacognostical, Phytochemical screening, *in-vitro and in-vivo* studies.

CONCLUSION

The ethanolic extract showed a significant sedative and hypnotic activity than the benzene and aqueous extracts. This may be due to one or more phytoconstituents like flavonoids present in the extract and it can be due to the isolated compound Malabaricone C with IUPAC name as (2,6-dihydroxyphenyl)-9-(3,4-dihydroxyphenyl)nonan-1-one..

Further research work has to be carried out to standardize the isolated molecule Malabaricone C and which can further screened for preclinical and clinical studies for sedative and hypnotic activity.

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Myristica malabarica: A comprehensive review

Prem Kumar Chelladurai and Radha Ramalingam

Abstract
The plant *Myristica malabarica* Linn belongs to the family Myristicaceae. This plant is endemic to India & found commonly in Western Ghats. It is an important medicinal plant commonly known as Malabar nutmeg, rampatri or Bombay mace. Different chemical constituents such as Malabaricones, Malabaricanal, Isoflavones and many other compounds are isolated and tested for pharmacological activity. *Myristica malabarica* is traditionally used for anti-ulcer, sedatives hypnotics, antimicrobial, nematicidal and as anti-inflammatory. This review has summarized to recent scientific findings of *Myristica malabarica's* phytochemistry, pharmacological activities of the plant.

Keywords: *Myristica malabarica,* Malabaricones, sedative, hypnotics, anti-ulcer, antimicrobial

Introduction
Myristica malabarica is commonly called as Malabar nutmeg or kaatuhjathi. It is native to India found widely in western ghat hills. *Myristica malabarica* seed and seed aril is used as spice in Indian foods. They enhance the taste and aromatic flavor of the food. Recent scientific studies proved their biological activity according to their traditional claims. They are now known to possess Gastroprotective, Antipromastigote, Antioxidant, Antifungal, Nematicidal, Antiproliferative, Leukemic and Solid tumor.

Plant Description
Myristica malabarica Lam. (Myristicaceae) is a perennial tree about 25m tall. It is endemic to India and found widely distributed in Western Ghats forest region. Now it is a vulnerable species according to IUCN red list. Hence it must be preserved.

Plant Taxonomy
Kingdom : Plantae
Super division : Angiosperms
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Mangoliales
Family : Myristicaceae
Genus : Myristica
Species : *Myristica malabarica*

Synonyms
Myristica dactyloides Wall
Myristica notha Wall
Myristica tomentosa J.Grah
Palala malabarica Kuntze

Common Names
Malabar nutmeg, false nutmeg, Bombay mace.

Vernacular Names
Hindi : Ramptri, Bambay-jayphal.
Kannada : Kanage, Doddajajikai.
Malayalam : Ponnampoovu, Kottappannu, Pathiripoovu, Panampalka.
Sanskrit : Bandhukapushpa, Gostani.
Tamil : Colaivenkai, Kattujatikkai.
Telugu : Adavijaikaya, Adividzajikaya.

Habit and Habitat
It is a large 15-25 mts tall perennial tree found evergreen forests upto 800mts. It is a swamp and lowland forest habitat tree. Large trunks with greyish black color. Flowering and fruiting season starts at feb-aug month. It is vulnerable species listed according to ICUN list due to drainage of swamp forests for agricultural purposes.

Ethano Medicinal Uses
The plant *Myristica malabarica* is traditionally used as medicine and spices in food. The aril is used as febrifuge, cooling, expectorant. In Ayurveda, aril is used for many conditions related to vata such as, fever, bronchitis, cough and burning sensation. The fat extracted from seed is used to treat indolent ulcers, analgesics and for rheumatism. In Ayurveda, for disorders in vata seed fat is used for myalgia, sprains and sores. The plant is also used for anti-inflammatory, Analgesic, anti-ulcer, sedative, hypnotic, and antimicrobial actions.

Phytochemical Constituents
The plant *Myristica malabarica* was found to contain various phytoconstituents such as Isoflavones, Diarylnonanoids which consists of Malabaricones A-D, Tannins and several other phytochemicals are also considered to be present in the plant. A brief study of phytoconstituents is needed for this plant.

Isoflavones
The heartwood of *Myristica malabarica* is found to have isolation of several isoflavones such as 7, 4'-dimethoxy-5-hydroxyisoflavone, biochanin A, prunetin and a rare alpha-hydroxydihydrochalcone.

Diarylnanoids
The plant is found to consist of different type of Diarylnonanoids which are commonly called as Malabaricones. *Myristica malabarica* contains four Malabaricones A, B, C and D. These malabaricones proved pharmacologically for various activity.

Tannins
From the investigation of the heart wood a tannin 1, 3-diarylpropanol is extracted.

Pharmacognostical Review
Morphology
Height: 25 m tall.

Trunk & Bark: Bark greenish-black, smooth, blaze reddish.

Branches and branchlets: Branches- horizontal, Branchlets-subtree, glabrous.
Exaduates: Sap red from cut end of bark, profuse.

Leaves: Leaves simple, alternate, distichous; Petiole 1-1.5cm long, caniculate above glabrous, lamina 9.5-22*3.7-10 cm, elliptic or elliptic oblong, apex acute or sub-acute, base acuteor attenuate, margin entire, glossy above, glabrous and glaucous beneath, coriaceous; mid rib raised above.

Inflorescence / Flower: Flowers unisexual, uroceolate, white; male flower numerous in number and smaller than female flowers, in auxillary cymes; female flowers in umbels, 5-6 flowered.

Fruit and seed: Capsule 5-7.5*1.8-3.5, oblong, pubescent; seed one, Aril covering the seed yellow and laciniate.

Pharmacological Review

Anti-cancer activity

Efficient and sensitive LC-MS/MS methods have been developed for the rapid screening and determination of bioactive compounds in fruit parts *Myristica malabarica.* Moreover, *in vitro* antiproliferative activity of these Myristica species was evaluated against five human cancer cell lines A549, DLD-1, DU145, FaDu and MCF-7 using SRB assay. Evaluation of *in vitro* antiproliferative activity revealed potent activity in *Myristica malabarica.*

Anti-diabetic activity

The extract of *Myristica malabarica* is assayed for *in vitro* insulin secretion studies on islets of langerhans at concentration of 1mg/ml. the results were promising and showed dose dependent insulin secretion. Regular use of these spices may prevent postprandial rise in glucose levels through inhibition of intestinal alpha-glucosidase and may maintain blood glucose level through insulin secretagogue action.

Anti-Fungal activity

Methanol extract of fruit rinds of *Myristica malabarica* exhibited potent antifungal activity against phytopathogenic fungi such as rice blast, tomato late blight, wheat leaf rust and red pepper anthracnose. The compounds exhibited antifungal activity are isolated and found out to be Malabaricones A, B and C.

Anti-Microbial activity

Aqueous and Methanolic extract of *Myristica malabarica* is assayed for their activity against multi-drug resistant *Salmonella typhi.* moderate Antimicrobial activity was shown by the methanolic extract of the plant *Myristica malabarica.*

Nematicidal activity

The fruit rinds of *Myristica malabarica* are extracted with methanol. The extract is subjected to nematicidal activity against the *Bursaphelenchus xylophilus* at a concentration of 1000 micro gram per ml and it is found to be very effective agaist the nematode. Studies show that malabaricones present in the plant *Myristica malabarica* is responsible for nematicidal activity.

Anti-Oxidant activity

The 1, 1-dipheny l-2-picrylhydrazyl (DPPH) assay of the ether, methanol, and aqueous extracts of the spice Myristica malabarica (rampatri) revealed the methanol extract to possess the best antioxidant activity. Column chromatography of the methanol extract led to the isolation of a new2-acylresorcinol and four known diarylnonanoids of which the diarylnonanoid, malabaricone C, showed the maximum DPPH scavenging activity. Malabaricone C could prevent both Fe (II)- and 2,2'-azobis (2-amidinopropane) dihydrochloride-induced lipid peroxidation (LPO) of rat liver mitochondria more efficiently.

Anti-Promastigote activity

This study was undertaken to evaluate the antileishmanial activity of the fruit rind of Myristica malabarica that is used as a spice and is also credited with medicinal properties. The antipromastigote activity of different extracts/fractions of M. malabarica and its constituent diarylnonanoids were evaluated in Leishmania donovani promastigotes (MHOM/IN/83/AG83) using the MTS-PMS assay. Preliminary screening of the ether extract (R1) with its crude methanol fraction (R2) and two fractions (R3 and R4) revealed that R2 had potent leishmanicidal activity (IC(50) 31.0 microg/mL). Methanol extract of M. malabarica, especially its constituent compounds, Mal A and Mal B, have promising antileishmanial activity.

Anti-ulcer activity

The healing activity of the methanol extract of the spice rampatri, Myristica malabarica, (RM) and omeprazole against indometacin-induced stomach ulceration has been studied in a mouse model. Treatment with RM (40 mg kg(-1) per day) and omeprazole (3 mg kg(-1) per day) for 3 days could effectively heal the stomach ulceration, as revealed from the ulcer indices and histopathological studies. Compared with the ulcerated group, treatment with RM and omeprazole for 3 days reduced the macroscopic damage score by approximately 72% and 76%, respectively ($P<0.001$), establishing the efficacy of RM. The healing capacities of RM and omeprazole could be attributed to their antioxidant activity as well as the ability to enhance the mucin content of the gastric tissues. Furthermore, RM improved the mucin level beyond the normal value, while omeprazole restored it to near normal.

Conclusion

Myristica malabarica a native plant of India has many medicinal properties. This present review is concerned mainly on the description, pharmacognostic features, phytochemical constituents and pharmacological activity. The major phytochemical constituents present in this plant which is

responsible for various pharmacological activity are Malabaricones A-D, and Isoflavones. The fruits, seeds, aril, bark and other parts of the plant extractives is found out to have various pharmacological activities like anti-cancer, nematicidal, anti-fungal, anti-bacterial, anti-ulcer, anti-oxidant, anti-diabetic and anti-promastigote. *Myristica malabarica* is a resource of various phytochemical constituents which can be used for medicinal proprety. Hence further investigation is required for this plant to discover various medicinally potential compounds for the benefit for mankind.

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CERTIFICATE

This is to certify that Mr. C. PREM KUMAR, M. Pharm., II Year, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-03, had submitted his Protocol (Part B Application) IAEC/MMC/10/2016 for the dissertation programme to the Animal Ethical Committee, Madras Medical College, Chennai-03.

TITLE: PHARMACOGNOSTICAL, PHYTOCHEMICAL EVALUATION AND SEDATIVE AND HYPNOTIC ACTIVITY ON THE SEED ARIL OF *Myristica malabarica* Lam.,

The Animal Ethical Clearance Committee experts screened his proposal Number: IAEC/MMC/10/2016 and have given clearance in the meeting held on 21.11.2016 at Anatomy demo hall III in Madras Medical College, Chennai-03. His study involves only Swiss albino mice.

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9th Aug 2016

CERTIFICATE

Certified that the specimen submitted for identification by C. Prem Kumar, M. Pharm 2nd year, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai - 600 003, is identified as aril of *Myristica malabarica* Lam.

Sasikala Ethirajulu

Sasikala Ethirajulu
Consultant (Pharmacognosy)

29/8/16

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