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Research Article

PHYTOCHEMICAL PROFILING OF *MYRISTICA FRAGRANS* SEED EXTRACT WITH DIFFERENT ORGANIC SOLVENTS

RANCY ANN THOMAS, KRISHNAKUMARI S*

Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.
Email: drskrishnakumari123@rediffmail.com

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ABSTRACT

Objective: The objective of this study was to evaluate the phytochemical constitution of dried seeds of *Myristica fragrans* using eight different solvent extracts such as methanol, ethanol, ethyl acetate, chloroform, petroleum ether, acetone, and aqueous (cold and hot).

Methods: Preliminary phytochemical screenings for various secondary metabolites were carried out. All the extracts were subjected to qualitative phytochemical screening and were analyzed for the presence of active constituents such as alkaloid, flavonoid, phenol, glycosides, and triterpenoids, etc.

Results: Qualitative analysis of the seed extracts confirmed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, phenols, anthraquinones, cardiac glycosides, coumarins, anthocyanin, chalcones, emodins, and triterpenoids.

Conclusion: The generated data has provided the basis for its wide uses as a therapeutic both in traditional and folk medicine. The observed phytochemical constituents in the seed extract may be associated with its high bioactive constituents that may serve as candidates to new drugs in the treatment and prevention of various human ailments.

Keywords: *Myristica fragrans,* Secondary metabolites, Phytochemical screening, Qualitative analysis.

INTRODUCTION

Man and animals depend on plants for their very existence.Our environment is characterized by richly diversified plant life. Plant diversity is composed of more than 500,000 botanical species. Plants constitute a vital component of the biodiversity as they play a key role in maintaining earth's environmental equilibrium and ecosystem stability. Herbal medicine is known to be the oldest form of healing, which was originated from ancient Greek as far back as 1600 BC [1]. It involves the use of plant materials such as flowers, bark, leaves, seeds or root to improve, maintain or restore health and wholeness [2].

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components [3]. The quantity and quality of phytochemicals present in plant parts may differ from one part to another. In fact, there is lack of information on the distribution of the biological activity in different plant parts essentially related to the difference in distribution of active compounds (or active principles) which are more frequent in some plant parts than in others [4].

Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently *en vogue* in parts of the world [5]. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region. Following such leads, plant parts are usually screened for phytochemicals that may be present. The presence of a phytochemical of interest may lead to its further isolation, purification and characterization. This is it used as the basis for a new pharmaceutical product. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. This therefore underscores the need to try as much solvents as possible in screening plant parts for phytochemicals.

Nutmeg (*Myristica fragrans*), whose seed is widely used as a spice, is a tropical, evergreen tree native to the Moluccas or Spice Island of Indonesia. Nutmeg has a characteristic pleasant fragrance and is slightly warm taste. It is also used as components of curry powder, teas and soft drinks, or mixed in milk and alcohol [6].

Scientific classification

Kingdom: Plantae
Order: Magnoliids
Family: Myristicaceae

The *M. fragrans* oil is used heavily in the perfumery and pharmaceutical industries. The oil is colorless or light yellow it contain numerous components of interest to the oleo chemical industry and is used as a natural food flavorings in baked food, syrups, beverages, and sweets. In traditional medicine nutmeg and nutmeg oil were used for illnesses related to the nervous and digestive system (Fig. 1) [7].

It has been estimated that phytochemical intake may be related to a reduction in cancer risk upwards of 20%. The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value [8]. For example, saponins have hypotensive and cardio depressant properties [9]. Glycosides are naturally cardio active drugs used in the treatment of congestive heart failure, and cardiac arrhythmia [10].The presence of saponins and glycosides in all the extracts might play a role in the cardio protective potential of nutmeg.

This study is based on the different solvent extraction of the seed of *M. fragrans* which were subjected to preliminary phytochemical screening and to analyze the presence of different secondary metabolites such as alkaloids, steroids, phenolics, tannins, glycosides, saponins, and flavonoid.

METHODS

Plant material

For this study the sample of fresh seeds of *M. fragrans* were collected from a farm of medicinal plants Kalamaserry, Ernakulam district

Kerala, India during the month of April 2014. Taxonomic identification of the plants was carried out with the help of Dr. V. S. Ramachandran, Professor, Bharathiar University, Tamil Nadu, India.

Sample processing
Plant sample was washed and shade dried at room temperature. The sample was then crushed into powder, using mechanical grinding machine, so as to enhance effective contact of solvent with sites on the plant materials. The dried and ground plant part was extracted with different solvents (methanol, ethanol, ethyl acetate chloroform, petroleum ether, acetone and aqueous [hot and cold]) by Soxhlet extraction. It was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using rotary evaporator.

Soxhlet extraction of essential oils
Raw plant materials consisting of seeds are put into distillation apparatus using Soxhlet apparatus. Weight of plant material was taken before loading in the Soxhlet apparatus and solvent is heated so that the steam passes through the plant material vaporizing the volatile compounds. The vapor flows through a coil where they condense back to liquid which is then collected in the receiving vessel.

Extractive values of plant sample
The extractive value or the yield percentage of the plant sample is calculated before and after the extraction process using the formula

Extract yield % = (W1/W2) × 100

Where,

W1 is net weight of powder in grams after extraction and,

W2 is total weight of powder in grams taken for extraction.

Preliminary phytochemical screening
The stock solution was prepared from each of the crude extracts such as ethanol, methanol, ethyl acetate, chloroform, petroleum ether, acetone and aqueous (hot and cold) 100 mg and dissolved in 10 ml of its own mother solvent. The obtained stock solutions were subjected to preliminary phytochemical screening.

RESULTS

Extractive values
The extractive values of ethanol, methanol, chloroform, petroleum ether, ethyl acetate, acetone and water extracts are given in Table 1. The highest extractive yield was found in the ethanolic seed extract.

Fig. 1: Myristica fragrans

Preliminary phytochemical screening
Chemical tests were carried out using aqueous extract to identify various constitutes using standard methods (Table 2). The results of the preliminary phytochemical screening carried out on eight different solvents revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, saponins, flavonoids, tannins, steroids supporting the reason for its wide range of biological activities as showed in Table 3. Tannins, phlobatannins, saponins, flavonoids, steroids and alkaloids were found to be universally present in *M. fragrans.* The ethanolic, methanolic and aqueous extract revealed the maximum presence of phytoconstituents whereas chloroform, petroleum ether and ethyl acetate extracts showed minimal amounts of phytoconstituents. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances.

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants

Table 1: Extractive value of *M. fragrans*

Type of extract	Yield (% w/w)
Ethanol	32.5
Methanol	28.5
Chloroform	1.95
Petroleum ether	2.75
Ethyl acetate	5.69
Acetone	12.60
Aqueous	19.80

M. fragrans: Myristica fragrans

Table 2: List of phytochemical tests performed

Constituents	Test
Alkaloids	Mayer's test
	Hager's test
Flavanoids	Shinoda test
Saponins	Foam test
	Froth test
Tannins	Gelatin test
Phenols	Ferric chloride test
	Lead acetate test
Anthraquinones	Borntrager'stest
Acids	Sodium bicarbonate test
Phlobtannins	Sulphuric acid test
	Ammonia test
Resins	Turbidity test
Coumarins	Sodium hydroxide test
Quinones	Acid test
Thiols	Sodium nitroprusside test
Terpenoids	Salkowski test
Triterpenoids	Liebermann Burchard test
Cardiac Glycosides	Keller-Killiani's test
	Legal's test
Oxalates	Acid test
Anthocyanins	Sulphuric acid test
Anthraracenoids	Borntrager's test
Emodins	Ammonium hydroxide test
Chalcones	Ammonium hydroxide test
Anthocyanosides	Sodium hydroxide
Gum and Mucilages	Swelling test
Carbohydrates	Molisch's test
	Benedict's test
Proteins	Biuret test
	Ninhydrin test
Volatile oils	Spot test
Fatty acids	Spot test
Steroids	Liebermann Burchard test
	Salkowski test

Table 3: Preliminary phytochemical screening of *M. fragrans* seed extract

Constituents	Ethanol extract	Methanol extract	Chloroform extract	Petroleum ether extract	Ethyl acetate extract	Acetone extract	Aqueous extract hot	Aqueous extract cold
Alkaloids	+++	+++	-	-	-	++	++	++
Flavanoids	++	+++	-	-	+	++	-	-
Saponins	++	+++	++	+++	++	+++	++	+++
Tannins	++	++	-	-	-	+	+	-
Phenols	+++	+++	-	-	-	++	-	+
Anthraquinones	+++	+++	-	-	-	+++	+	++
Acids	+++	-	-	-	-	-	++	+
Phlobtannins	++	-	-	-	-	-	-	+
Resins	++	++	++	+	-	++	++	+++
Coumarins	+++	+++	-	-	-	+++	+	++
Quinones	+++	+++	++	++	+	+++	+++	+++
Thiols	+	+	+	++	+	+	+	+
Terpenoids	+++	+++	-	++	-	+	-	+
Triterpenoids	-	-	-	-	-	-	-	+
Cardiac glycosides	+++	+++	-	++	-	+	-	++
Oxalates	-	-	-	-	-	-	-	-
Anthocyanins	+++	+++	-	++	-	+	-	++
Anthraracenoids	-	-	-	-	-	-	-	-
Emodins	++	+	+	-	-	+	++	+
Chalcones	++	+	+	+	+	+	++	+
Anthocyanosides	++	+++	-	-	-	++	+	+
Gum and mucilages	++	++	++	-	-	++	++	+
Carbohydrates	+++	+++	+++	+++	+++	+++	+++	++
Proteins	+++	++	-	-	+++	+	++	++
Volatile oils	+++	+++	++	++	++	+	-	++
Steroids	+++	++	+	-	-	+	++	+
Fatty acids	+++	+++	+++	+++	+++	++	++	++

Key: +: Trace, ++: Present, +++: Excess, - Absent

serve as defense mechanism against predation by many microorganism, insects and herbivores. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites [12]. It may be concluded that these medicinal plants are very useful. These plants may be used to cure some common and other various diseases.

DISCUSSION

Most of the traditional knowledge about medicinal plants was in the form of oral knowledge. There is no uniform or standard procedure for maintaining the inventory of these plants and the knowledge about their medicinal properties.

Therefore, it is necessary that such procedures to be documented and studied for systematic regulation and widespread application. The leads for a significant number of modern synthetic drugs have originated from isolated plant ingredients since the search for new entities begins from either derivatizing the existing drug or from traditional medicinal system. It is very important to undertake phytochemical investigations along with biological screening to understand therapeutic dynamics of medicinal plants and also to develop quality parameters.

In the phytochemical analysis different polarity of phytoconstituents were sorted out from the coarsely powdered seeds of *M. fragrans* (Houtt.) by using solvents like ethanol, chloroform, ethyl acetate, petroleum ether, acetone and methanol by successive extraction using Soxhlet apparatus.

Successive extractive values revealed the solubility and polarity particulars of the metabolites in the plant. Ethanolic extract showed high extractive yield 32.5% w/w when compared to other extracts.

Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in each extract. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their qualitative estimation and separation of pharmacologically active chemical compounds.

The phytochemical screening in the present study has revealed the presence of triterpenoids, steroids, glycosides, flavonoids, tannins, triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrate and steroids in the seed extract (Table 2). Further the presence of different phytoconstituents in the three different extracts may be responsible for the therapeutic properties of nutmeg.

The significance of medicinal plants is directly linked to the wide range of chemical compounds synthesized by the various biochemical pathways. These compounds are classified as secondary plant products, because they are not much related to the plant's survival. Previously, researchers took many of these compounds to be simply waste products of metabolism, but they are now known to possess important functions.

One major category of such compounds is alkaloids. Although they vary greatly in their chemical structures, alkaloids have several common characteristics: They possess nitrogen (most are derived from a few common amino acids), and are alkaline (basic), but have non- basic forms such as quaternary compounds and N-oxides. The alkaloid extracts obtained from medicinal plant species have multiplicity of host-mediated biological activities, including antimalarial, antimicrobial, anti-hyperglycemic, anti-inflammatory, and pharmacological effects [13,14].

Anthraquinones are a class of natural products encompassing several hundreds of compounds, differing in the nature and positions of substituent groups. Many anthraquinones have potential therapeutic value, since antimicrobial, insecticidal, antitumor, anti-congestive, hypotensive, and sedative properties have been assigned to these compounds [15,16].

The use of cardiac glycoside containing plants for medicinal purposes was first reported in ancient texts more than 1500 years ago.

Their positive inotropic effects help suppress the active counter-transportation of Na+ and K+ across the cell membrane, leading to an increase in the intracellular Na+ concentration, a decrease in the intracellular K+ concentration, and a consequent increase in cardiac contraction [17]. Furthermore, studies have suggested that cardiac glycosides target cancer cells selectively and have a significantly lower mortality rate [18].

Chalcones and its derivatives have attracted increasing attention due to numerous pharmacological applications. They have displayed a broad spectrum of pharmacological activities, among which antimalarial [19], anticancer [20,21], antiprotozoal (anti-leishmanial and anti-trypanosomal) [22], anti-inflammatory [23], antibacterial [24], anti-filarial [25], antifungal [26], antimicrobial [27], larvicidal [28], anticonvulsant [29], antioxidant [30] activities have been reported. They have also shown inhibition of the enzymes, especially mammalian alpha-amylase [31], cyclooxygenase (COX) [32] and monoamine oxidase [33]. They have shown antimitotic activity too [34].

Coumarins have been reported to exhibit antioxidant, analgesic, anti-inflammatory and anti-mutagenic properties [35].

Terpenoids and tannins are attributed for analgesic and anti-inflammatory activities. Apart from this tannins contribute property of astringency i.e. faster the healing of wounds and inflamed mucous membrane [36].

Emodins forms the basis of purgative anthraquinones derivatives and from ancient times has also been widely used as a laxative compound [37]. Recent studies have shown that emodin also exhibits numerous other biological activities, which affect the immune system, vasomotor system and the metabolic processes [38-40].

Flavonoids possess many pharmacological activities like anti-ulcer, anti-ageing, anti-bacterial, anti-oxidant, anti-fungal, anti-inflammatory, anti-diabetic, anti-hepatotoxic, anti-allergic anti-cancer, anti-tumor and vasodilator properties. Furthermore flavonoids show potential vitamin C sparing activity and activities of lipoxygenase, COX, protein kinase C, tyrosine kinase, etc. Majority of flavonoids are powerful antioxidants that help neutralize harmful free radicals and prevent oxidative stress, which damage cells and deoxyribonucleic acid, and which can lead to aging and degenerative diseases like cancer and Alzheimer's or Parkinson's disease [41-47].

Plant gums and exudates are now screened for their use as pharmaceutical adjuvants. Mucilages of different origins are also used in conventional dosage forms of various drugs for their binding, thickening, stabilizing and humidifying properties in medicine. Gums, resins and latexes are employed in a wide range of food and pharmaceutical products and in several other technical applications. In the pharmaceutical industry they are used as binding agents in tablets and as suspending and emulsifying agents in creams and lotions; some have specific applications in the dental and medical fields [48-49].

Biological thiols found in plants can also function as an antioxidant, an anti- mutagen, and an anti-carcinogen [50].

CONCLUSIONS

The presence of phytoconstituents makes the plant useful for treating different ailments and has a potential of providing useful drugs for human use. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases [51]. In the present study, we have found that most of the biologically active phytochemicals were present in the ethanolic, aqueous and methanolic extracts of *M. fragrans* seeds. Since the ethanolic extract of the seed contains more constituents it can be considered beneficial for further investigation. The medicinal properties of *M. fragrans* seed extract may be due to the presence of above mentioned phytochemicals.

Phytochemicals found present in seed extracts of *M. fragrans* indicates their potential as a source of principles that may supply novel medicines. Further studies are therefore suggested to ascertain their pharmacological activities. Furthermore, isolation purification and characterization of the phytochemicals found present will make interesting studies.

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