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PHYTOCHEMICAL QUANTIFICATION OF PRIMARY AND SECONDARY METABOLITES OF *MYRISTICA FRAGRANS* (H.) ETHANOLIC SEED EXTRACT

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ABSTRACT

The therapeutic use of herbs is as old as human civilization and has evolved along with it. A vast majority of people on this planet still rely on their indigenous system of medicine and use herbal drugs. The Indian and Chinese systems of medicine are well established with written records going back around 3000 years. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets, including cancer, malaria, cardiovascular diseases and neurological disorders. Interest in herbal drugs and natural medicine is undergoing a renaissance at the present time. The medicinal properties of plants are due to the presence of active principles such as alkaloids, phenolics, tannins and flavonoids which constitute of many pharmacologically active compounds. The aim of the present study was to quantify the presence of a few primary and secondary metabolites in the nutmeg seeds.The results of phytochemical screening of ethanolic seed extract of *Myristica fragrans* revealed the presence of primary metabolites like carbohydrates, proteins and amino acids and secondary metabolites such as alkaloids, flavonoids, tannins and phenols. The high level of primary metabolites in the sample reveals its nutritive value. These secondary metabolites could be responsible for the observed medicinal properties of *Myristica fragrans* by traditional herbalists.

KEYWORDS: **Primary and secondary metabolites, medicinal properties, *Myristica fragrans*,**

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INTRODUCTION

In the ancient India, medicinal plants were used to prevent various critical diseases. The plant kingdom is an important source of herbal drugs. Even in recent years, there has been an increasing awareness about the importance of medicinal plants. Generally, herbal drugs are easily available, safe, less expensive, efficient, and rarely have side effects. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs[1]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and phenols. The bio-active phytocompounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure functions. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [2].Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases [3] and have possible benefits to the humanity. Botanical medicines or phytomedicines refer to the use of seeds, fruits, leaves, bark, roots or flowers of any plant for medicinal purposes by a significant number of people. Knowledge of the chemical constituents of plants is desirable because such information will be valued for synthesis of complex chemical substances [4-6]. *Myristica fragrans* Houtt, traditionally known as Jatiphal and Javitri in India, belongs to the family Myristicaceae. It mainly produces two spices, nutmeg seed and mace the thick fiber like red aril on the kernel [7]. Nutmeg the seeds of *M. fragrans* have been used for the treatment of heart ailments in Ayurvedic system of Medicine. In Indian households it is used not only as an aromatic substance, but also for flavor, spice and as a condiment [8].The chemical constituents of *Myristica fragrans* have been investigated by scientists from various disciplines for hypolipidemic and hypocholesterolemic effects, antimicrobial, antidepressant, aphrodisiac, memory-enhancing, antioxidant, and hepatoprotective properties [9]. In traditional medicine, the seed kernel (nutmeg) is widely used as carminative, astringent, hypolipidemic, antithrombotic, antiplatelet aggregation, antifungal, aphrodisiac [10], treating flatulence, nausea, and dyspepsia [11]. Mace is widely used as a flavoring agent, a hair dye, and a folk medicine. It also possesses anti -papilloma genic, anti-carcinogenic[12], and anti-inflammatory activities [13].Plant cells produce two types of metabolites. Primary metabolites are involved directly in growth and metabolism, viz. carbohydrates, lipids and proteins. Primary metabolites are produced as a result of photosynthesis and are additionally involved in cell component synthesis. Most natural products are compounds derived from primary metabolites such as amino acids, carbohydrates and fattyacids and are generally categorized as secondary metabolites. Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity viz. alkaloids, phenolics, essential oils, terpenes, flavonoids, lignins, tannins, etc. These secondary metabolites are the major source of pharmaceuticals, food additives, fragrances and pesticides [14-17].Vitamins are organic compounds required as vital nutrients in tiny amounts by an organism. Vitamins serve as biocatalysts in many chemical reactions as well as precursors to various body factors. They also required for a variety of biological processes such as mental alertness e.g niacin; resistance to infections e.g. vitamin C. Vitamin A is necessary in vision , gene transcription [18, 19], immunity, dermatology [20], growth and development [21] and so on. Thiamine derivatives and thiamine dependent enzymes are present in all cells of the body, thus a thiamine deficiency would seem to adversely affect all of the organ systems. However, the nervous system and the heart are particularly sensitive to thiamine deficiency because of their high oxidative metabolism[22]. In the present work, quantitative phytochemical analysis was conducted on *Myristica fragrans* with the aim of

identifying and determining the actual phytochemicals and quantity of each constituent present in the seeds of the plant

MATERIALS AND METHODS

Plant material

Fresh seeds of *Myristica fragrans* were collected from an Ayurveda botanical park in Ernakulam, Kerala on April 2014. Plant sample was washed and shade dried at room temperature. The dried and ground plant part extracted with ethanol by a Soxhlet apparatus.

Extraction of essential oil

Raw plant materials consist of powdered seed and are put into distillation unit using a Soxhlet apparatus. Weight of plant material (5 – 10 g) was taken before loading in the Soxhlet apparatus and ethanol is heated so that the steam passes through the plant material vaporizing the volatile compounds. The vapour flows through a coil where they condense back to liquid which is then collected in the receiving vessel.

Determination of primary metabolites

Primary metabolites are directly involved in growth and development. Primary metabolites are of prime importance and essentially required for growth of plants.

1.Determination of total soluble Carbohydrates

The total soluble Carbohydrate content was determined according to the method described by Hedge and Hofreider, 1962 [23].1 ml of sample was mixed with 4 ml of Anthrone reagent. Incubated in boiling water bath for 8 minutes, after which the absorbance was read at 630 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg / g sample

2. Determination of Proteins

Protein content was determined according to the method of Lowry et al., 1951 [24].1 ml of sample was mixed with 0.5 ml of 0.1 N NaOH and 5 ml of alkaline coper reagent,incubated the mixture at room temperature for 30 minutes.Added 0.5 ml of Folin-Ciocalteau reagent and incubated again for 10 minutes at room temperature. Absorbance was read at 660 nm against a reagent blank.The analysis was performed in triplicates and the results were expressed mg / g sample.

3. Determination of Total free amino acids

Total free Amino acid (Ninhydrin method) was determined according to the procedure given by Moore and Stein, 1948 [25] .1 ml of the sample was mixed with 1 ml of Ninhydrin reagent in a test tube.Tubes were kept in boiling water bath for 20 minutes and then added 5 ml of diluent (equal volume of water and n-propanol).Incubated at room temperature for 15 minutes and absorbance were read at 570 nm against a reagent blank.The analysis was performed in triplicates and the results were expressed as mg / g sample.

4. Determination of Vitamins

Vitamins in Myristica fragrans were estimated by standard methods. Vitamins have diverse biochemical functions and thus the necessity for its analysis.

Table I
List of protocols followed

Vitamins	References
Vitamin A	Nedd and Pearson, 1963 [26]
β carotene	Nedd and Pearson, 1963 [26]
Vitamin B_1	Sadasivam and Manickam, 1996 [27]
Vitamin C	Omaye et al., 1979 [28]
Vitamin E	Varley at ao., 1981 [29]

Quantitative determination of secondary metabolites

Drug discovery from the medicinal plants has played significant role in the treatment of various diseases and indeed, most new clinical applications of plants secondary metabolites and their derivatives over the last century. Secondary metabolites are important mediators of ecological interactions between plants and their environment.

1. Determination of Total phenols

Total phenol content were estimated in the ethanolic extract by the procedure given by Bray and Thorpe, 1954 [(30)], Folin-ciocalteau method.To 1 ml of sample added 0.5 ml of Folin-ciocalteau reagent and incubated at room temperature for three minutes.After three minutes 2 ml of 20% Na_2CO_3 was added, mixed well and incubated the tubes in boiling water bath for 1 minute.Cooled rapidly and read absorbance at 650 nm against reagent blank.The analysis was performed in triplicates and the results were expressed as mg / g sample.

2. Determination of Flavonoids

Flavonoids in ethanolic extract of *Myristica fragrans* was estimated by the method proposed by Jia et al., 1954 [31].1 ml of the was mixed with 0.075 ml of 5% Sodium nitrite solution and incubated at room temperature for 10 minutes.Then added 10% aluminum chloride and incubated at room temperature for 6 minutes. Then added 1 N NaOH and absorbance was read at 510 nm against a reagent blank.The analysis was performed in triplicates and the results were expressed as mg catechin equivalent/ g sample.

3. Determination of Tannins

Estimation of tannins in the extract was done by the procedure given by standard methods of Bray and Thorpe, 1954 [30].1 ml of the sample was mixed with 5 ml of vanillin hydrochloride reagent and incubated at room temperature for 20 minutes.Absorbance was read at 500 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as catechin equivalents.

Statistical Analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation (SD).

RESULTS AND DISCUSSION

Biologically active compounds contain a remarkably diverse assay of organic compounds and the carbohydrates are not only the first formed organic compounds in the plants as a result of photosynthesis but, also a major source of energy. Not only this, all the biochemical compounds are directly/indirectly derived from them for the important framework or they also modify the physico- chemical characters of other groups of compounds by combining with them[31-32]. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Plants are rich sources in flavoring, fragrances, insecticides; sweeteners and natural dyes [33]. The primary metabolites present in the sample are represented in the table: 1. Carbohydrates are one such group of carbon compounds which are essential to life. Almost all organisms use carbohydrates as building blocks of cells and as a matter of fact, exploit their rich supply of potential energy to maintain life[34]. The amount of carbohydrate was observed in the seed ethanolic extract was found to be 2.17 ±0.035. Proteins occur throughout the plant cells, both in extrinsic and intrinsic, simple and conjugated forms. In many plant species the exhibited bioactivity, viz. antiviral and others has been attribute to the proteinaceous substances present in their tissues. As a result, a study on the quality and quantity of proteins has been undertaken in the plant sample. In the present investigation, therefore, the selected plant sample was assessed for the total protein content. Higher levels of proteins were observed in the seed sample. Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base

bioactive compound could also be isolated in future [35]. Total levels of protein were found to be 4.57±0.024 mg/g. An organic chemical compound (or related set of compounds) is called a vitamin when the organism cannot synthesizethe compound in sufficient quantities, and must be obtained through the diet; thus, the term "vitamin" is conditional upon the circumstances and the particular organism. For example, ascorbic acid (vitamin C) is a vitamin for humans, but not for most other animal organisms. Supplementation is important for the treatment of certain health problems. Vitamins have diverse biochemical functions. Some, such as vitamin D, have hormone-like functions as regulators of mineral metabolism, or regulators of cell and tissue growth and differentiation (such as some forms of vitamin A). Others function as antioxidants (e.g., vitamin E and sometimes vitamin C) .The largest number of vitamins, the B complex vitamins, function as precursors for enzyme cofactors, that help enzymes in their work as catalysts in metabolism. In this role, vitamins may be tightly bound to enzymes as part of prosthetic groups: For example, biotin is part of enzymes involved in making fatty acids. They may also be less tightly bound to enzyme catalysts as coenzymes, detachable molecules that function to carry chemical groups or electrons between molecules. For example, folic acid may carry methyl, formyl and methylene groups in the cell. Although these roles in assisting enzyme-substrate reactions are vitamins' best-known function, the other vitamin functions are equally important [28]. Secondary metabolites have evolved in nature in response to the needs and challenges of the plant environment. Nature has been carrying out its own combinatorial chemistry for over three billion years [36]. The total number of natural products produced by plants has been estimated at over500,000[37].The quantitative phytochemical estimation present in *Myristica fragrans* studied showed that the seeds are very rich in alkaloids, tannins, phenolics and flavonoids (Table 2). Alkaloids have been known to humans for several centuries. They are a diverse group of low-molecular-weight, nitrogen-containing compounds found in about 20% of plant species. The potent physiological activity of many alkaloids has also led to their use as pharmaceuticals, stimulants, narcotics and poisons. Alkaloids currently in clinical use include the analgesics morphine and codeine, the anticancer agent vinblastine etc. The plant alkaloids like caffeine in tea and coffee and nicotine in all preparations (smoking, chewing) of tobacco are widely consumed daily [38]. Flavonoids have two benzene rings attached by a propane unit and are derived fromflavones. They are found throughout the plant kingdom. They function as antimicrobial, anti-insect compounds and mostly exhibit estrogenic, antioxidant and anticancer properties [39]. The presence and large amount of tannins also confirms its astringent property. This compound can also be effective in protecting the kidneys [39]. Tannins have also shown potential antibacterial and antiviral effects [40. 41]. Polyphenols are the most widely distributed class of plant secondary metabolites and several thousand different compounds have been identified. Polyphenols play many different roles in plant biology and human life, including UV protective agents, defensive compounds against herbivores and pathogens, contributors to plant colors, contributors to the taste of food and drink, and pharmaceuticals [42,43]. Several papers published in the 1960s showed that polyphenols could inhibit the activity of digestive enzymes and/or precipitate nutritional proteins [44]. Nutmeg is rich in many vital B-complex vitamins, including vitamin C, folic acid, riboflavin, niacin, vitamin A and many flavonoid anti-oxidants like beta-carotene and cryptoxanthin that are essential for optimum health. The amount of vitamins were analyzed and represented in table: 3. Ascorbic acid was found to be majorly present in the sample compared to the other vitamins. Thus, approving nutmeg's wound healing and immunity booster activities.

Table I
Primary metabolites in Myristica fragrans

Primary Metabolites	Quantity (mg/g)
Carbohydrates	2.17 ±0.035
Protein	4.57±0.024
Amino acids	1.80±0.02

Values are expressed by mean ± SD of 3 Samples

Table II
Secondary metabolites in Myristica fragrans

Secondary Metabolites	Quantity (mg/g)
Alkaloids	3.17±0.09
Flavonoids	4.89±0.02
Tannins	2.23±0.07
Phenols	1.31±0.06

Values are expressed by mean ± SD of 3 Samples

Table III
Vitamins in *Myristica fragrans*

Vitamins	Quantity (mg/g)
Vitamin A	0.39 ± 0.03
β carotene	0.28 ± 0.02
Vitamin B_1	0.18 ± 0.02
Vitamin C	0.67 ± 0.06
Vitamin E	0.21 ± 0.03

Values are expressed by mean ± SD of 3 Samples

The therapeutic potential of herbs has been well recognized by various indigenous systems of medicine[45]. Besides their therapeutic use, herbs are disease preventers and also used as cosmetics, dietary supplements and for reducing obesity. The seed extract has a high carbohydrate content and can be concluded that Myristica fragrans is a very good source of energy. The presence of higher protein level in the sample hints towards their possible increase in food value or that a protein base bioactive compound could also be isolated in the future{46}. Thus, the results obtained in the present study indicates nutmeg has the potential to act as a source of useful drugs because of presence of various phytochemical components such as carbohydrate, protein, alkaloids, phenols, flavonoids and tannin. The results are very much encouraging, but scientific validation is necessary before being put into practice.

Conflict of interest

We declare that we do not have conflict of interest.

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