

## Research Article



## Determination of Amino Acid and Lipid Contents in an Indian Spice – *Myristica fragrans*

Rancy Ann Thomas<sup>1</sup>, S. Krishnakumari<sup>\*</sup>

<sup>1</sup>PhD Scholar, Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore, Tamil nadu, India.

<sup>\*</sup>Guide and Supervisor, Associate Prof in Biochemistry, Dept of Biochemistry, Kongunadu Arts and Science College, Coimbatore, India.

<sup>\*</sup>Corresponding author's E-mail: [drkrishnakumari123@rediffmail.com](mailto:drkrishnakumari123@rediffmail.com)

Accepted on: 03-04-2015; Finalized on: 31-05-2015.

### ABSTRACT

Spices are used to flavor food and beverages worldwide. They are indispensable in the daily diet of Indians. Apart from enhancing taste, spices are known to have medicinal values. They are used in many medicinal preparations for the treatment of several body ailments, especially for problems of the digestive system. The present study deals with the estimation of amino acid and lipid contents present in *Myristica fragrans* in order to extend investigations on its pharmacological properties.

**Keywords:** *Myristica fragrans*, spices, medicinal value, lipid and amino acid content.

### INTRODUCTION

Traditional medicine practice entails the use of natural resources like plants, animals, water and minerals. The use of plants as an alternative for the treatment of renal, gastrointestinal, rheumatism, liver, anti-diabetes, amongst other diseases, has increased over the years. Spices used in Indian cooking have a long history of use as medicines to prevent and treat diseases. Many studies have confirmed that spices are useful medicines, but the major challenge is to provide scientific evidence and plausible mechanisms for their therapeutic responses.<sup>1,2</sup>

The importance of amino acids cannot be over emphasized as they form the basement or building block of the molecular structure of the important and very complex class of compounds known as the protein. They are also required for synthesis of metabolic products, including: purines and pyrimidines for nucleic acid synthesis, haem, thyroid hormones, and as well as melanin.<sup>3</sup>

Amino acids are essential in the synthesis of proteins and precursors in the formation of secondary metabolism molecules that participate in cell signaling, gene expression and homeostasis regulation, protein phosphorylation, synthesis of hormones and antioxidant capacity. Also, amino acids participate in various physiological processes such as skeletal muscle function, atrophic conditions, sarcopenia and cancer.<sup>4-7</sup>

Drought stress and saline are conditions that favor the accumulation of products of primary metabolism as osmolytes, amino acids and reducing sugars. Also, the plants are adapted to several stress conditions such as high and low temperatures, high salt concentrations, exposure to heavy metals, ultraviolet radiation and drought stress as result of their continuous evolution. The response of the plants results in the activation of mechanisms involved in the synthesis and accumulation

of secondary metabolites responsible for the beneficial properties attributed to medicinal plants.<sup>8</sup>

Lipids are diverse group of fatty substances found in all living organisms which do not dissolve in water but are soluble in alcohol, ether and other organic solvents.

Lipids are essential to life and that they are essential fats that play a very important role in the human body. They help our body to run smoothly in many ways, help the brain to function well, joint mobilization, energy production and also help the body absorb vitamins, coats the cells with lubrication therefore providing protective barrier, acts as messengers in the body (internal cellular communication), work to regulate steroidal hormones.<sup>9</sup>

Nutmeg has its origins in the Spice Islands of Indonesia, formerly known as the Spice islands, is also cultivated in the Caribbean, south India, Sri Lanka, Sumatra, and Malaysia. It has been widely popular in Europe and India for its flavoring and medicinal properties. The oil of nutmeg has also been used for medicinal purposes and it is this fraction of the nutmeg which contains the pharmacologically active components. For many years, control of bacterial infections by inhibiting microbial growth has been a primary approach of antimicrobial chemotherapy. About 30-55% of the seed consists of oils and 45-60% consists of solid matter including cellulose materials. There are two types of oils: The "essential oil of nutmeg" also called the "volatile oil" accounts for 5-15% of the nutmeg seed and the "fixed oil of nutmeg" sometimes called "nutmeg butter" or expressed oil of nutmeg accounts for 24-40% of the nutmeg seed. The relative percentages of the different components will vary depending on the geographical origin of the nutmeg.<sup>10-13</sup>

However, few studies focus on primary metabolites in plants naturally adapted to tolerate prolonged periods of drought stress/saline and the relationship with their therapeutic properties. To assess the relationship between the production of primary metabolites and their



possible therapeutic properties, we analyzed the amino acid and lipid profile of *Myristica fragrans* used in traditional medicine. The study of compounds generated by plants as a result of defense mechanisms permit understanding of the molecular mechanism involved in their medicinal properties.

## MATERIALS AND METHODS

### Plant Material

For the present study the sample of fresh seeds of *Myristica fragrans* were collected from a farm for 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. 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 vapour flows through a coil where they condense back to liquid which is then collected in the receiving vessel. The dried and ground plant part was extracted with ethanol by Soxhlet extraction. It was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using rotary evaporator.

### Sample Preparation

The extract received as a result of Soxhlet extraction is further condensed using a rotary evaporator. The sample was extracted using ethanol and was further used for analysis of lipids and amino acid contents.

### Quantitative Estimation of Lipids

Lipids are an essential constituent of all plant cells. The vegetative cells of plants contain 5 to 10% lipid by dry weight, and almost all of this weight is found in the membranes. Membrane lipids are important for improvement of photosynthesis against high temperature stress and improved photosynthesis means improved stress tolerance as well. In the present study free fatty acids, total cholesterol, phospholipids, triglycerides were estimated.<sup>14</sup>

**Table 1:** Protocols Involved

Parameters	References
Free fatty acids	Horn and Mehanan, 1981 <sup>15</sup>
Total cholesterol	Parekh and Jung, 1970 <sup>16</sup>
Phospholipids	Rouser, 1970 <sup>17</sup>
Triglycerides	Rice, 1970 <sup>18</sup>

### Quantitative Estimation of Amino acids

The amino acids have several roles in plants, for example they act as osmolytes, detoxify heavy metals, and regulate ion transport, stomatal opening, affect synthesis and activity of enzymes, gene expression and redox homeostasis. Amino acids such as Tryptophan, Methionine, Histidine, Proline and Arginine were quantitatively estimated.<sup>19</sup>

**Table 2:** Protocols Involved

Parameter	References
Tryptophan	Sadasivam and Manickam, 1996. <sup>20</sup>
Methionine	Sadasivam and Manickam, 1996. <sup>20</sup>
Histidine	Kapeller and Adler, 1933. <sup>21</sup>
Proline	Sadasivam and Manickam, 1996. <sup>20</sup>
Arginine	Sakaguchi, 1925. <sup>22</sup>

### 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

Lipids are the major form of carbon storage in the seeds of many plant species. Lipids are the most effective source of storage energy, function as insulators of delicate internal organs and hormones and play an important role as the structural constituents of most of the cellular membranes.

Dried nutmeg is used as spice. The essential oil (also called volatile oil) and the oleoresin are the major products of interest from the spice. Nutmeg is a stimulant, carminative, astringent and aphrodisiac. It is used in tonics and electuaries and forms a constituent of preparations for dysentery, stomachache, flatulence, nausea, vomiting, malaria, rheumatism, sciatica and early stages of leprosy. Higher doses have a narcotic effect. It is chewed for masking foul breath. It also prevents dental caries.<sup>23</sup>

The role played by accumulated amino acids in plant varies from acting an osmolyte, regulation of ion transport, modulating stomatal opening and detoxification of heavy metals.

Amino acids also affect synthesis and activity of some enzymes, gene expression and redox homeostasis. Free amino acids and polyamines take part in several metabolic processes and they are involved in the protection against abiotic stresses.

Plants synthesize amino acids from the primary elements, the carbon and oxygen obtained from air and hydrogen from water in the soil, forming carbon hydrate by means of photosynthesis and combining it with the nitrogen which the plants obtain from the soil, leading to synthesis of amino acids, by collateral metabolic pathways.<sup>24</sup>



The results obtained from the present study are represented in Table 3 and Table 4.

**Table 3:** Quantitative Estimation of Lipids

Lipids	Amount (mg/g)
Triglycerides	1.82 ± 0.03
Total cholesterol	0.06 ± 0.002
Free fatty acids	3.13 ± 0.03
Phospholipids	0.96 ± 0.05

Values are expressed by mean ± SD of 3 Samples

In the present study ethanolic extract of *Myristica fragrans* shows higher amount of Free fatty acids (3.13 ± 0.03 mg/g) followed by triglycerides (1.82 ± 0.03 mg/g). Fatty acid is found in every plant cell and is essential for growth.

As part of complex lipids, fatty acids are also important for thermal and electrical insulation, and also for mechanical protection. Triglycerides make up the chemical structure of all vegetable oils and fats found in nature. It acts as energy reserve when stored as adipose tissue also acts as insulator, shock protection.

**Table 4:** Quantitative Estimation of Amino acids

Amino acids	Amount (mg/100g)
Tryptophan	0.82 ± 0.05
Methionine	0.18 ± 0.03
Histidine	1.04 ± 0.07
Proline	0.29 ± 0.04
Arginine	2.04 ± 0.08

Values are expressed by mean ± SD of 3 Samples

## CONCLUSION

Nutmeg is potentially used for supplement and pharmaceutical exploration due to its high antioxidant properties, economical price and readily available.

The use of aqueous ethanol (80%) is recommended as it is non-toxic to human.

The present study indicated that nutmeg is rich in important food nutrients compared to other oil seeds and nuts. However, the present investigation showed that the studied plant is potentially a good source of traditional medicine.

The nutritional analysis of the indigenous edible plant by chemical means informs one only of the potential values of this food to those populations who rely upon them as staples or supplements to their diet.

The next step is to assess the bioavailability of the essential nutrients in this spice; such studies are contemplated. These studies will focus on the digestibility of the proteins and lipids in these plants and on the possible presence of anti-nutrients, such as metal chelators (e.g., phytates, oxalates) and protease inhibitors.

## REFERENCES

- Bautista Cruz A, Arnaud Viñas MR, Martínez Gutiérrez GA, Sánchez-Medina PS and Pacheco RP, The traditional medicinal and food uses of four plants in Oaxaca, Mexico, *J Med Plants Res*, 5, 3404-3411, 2011.
- Retelny VS, Neuendorf A and Roth JL, Nutrition protocols for prevention of cardiovascular diseases, *Nutr. Clin. Pract*, 23, 468–476, 2008.
- Zarraga IGE and Schwarz ER, Impact of dietary patterns and interventions of cardiovascular health, *Circulation*, 114, 961–973, 2006.
- Pérez-Urria CE and Avalos García A, *Metabolismosecundario de plantas*, REDUCA, 2, 119-145, 2009.
- Cuin TA and Shabala S, Amino acids regulate salinity-induced potassium efflux in barley root epidermis, *Planta*, 225, 753-761, 2007.
- Wu G, Amino acids: metabolism, functions, and nutrition, *Amino Acids*, 37, 1-17, 2009.
- Nicastro H, Artioli GG, dos Santos Costa A, Solis MY, da Luz CR, Blachier F and Lancha AH. An overview of the therapeutic effects of leucine supplementation on skeletal muscle under atrophic conditions, *Amino Acids*: 1-14, 2011.
- Dioguardi FS, Clinical use of amino acids as dietary supplement: pros and cons, *J Cachexia Sarcopenia Muscle*: 1-6, 2011.
- Vivanco JM, Cosio E, Loyola-Vargas VM, Flores HE, *Mecanismosquímicos de defensaenlasplantas*, Investigación y Ciencia, 341, 68-75, 2005.
- Vanisree M, Lee CY, Lo SF, Nalawade SM, Lin CY and Tsay HS. Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures, *Bot Bull Acad Sin*, 45, 1-22, 2004.
- Ochulayi P and Anekwe GE, Lipid composition of medicinal plants: Portulacaoleracea and Ficusthonningi, *African Journal of Food Science*, 7(5), 76-79, 2013.
- Barceloux DG, *Medical Toxicology of Natural Substances: foods, fungi, medicinal herbs, plants, and venomous animals*, John Wiley and Sons Inc. 2008.
- Prakash Ekta and Gupta Dwijendra K, Cytotoxic Activity of Ethanolic Extract of *Myristica fragrans* (Houtt) Against Seven Human Cancer Cell Lines, *Universal Journal of Food and Nutrition Science*, 1(1), 1-3, 2013.
- Murakami Y, Tsuyama M, Kobayashi Y, Kodama H and Iba K., Trienoic fatty acids and plant tolerance of high temperature, *Science*, 287, 476–479, 2000.
- Horn WT and Menahan L.A, A sensitive method for determination of free fatty acids in plasma, *J Lipids Res.*, 122, 377–381, 1981.
- Parekh A.C and Jung D.H, Cholesterol determination with ferric acetate–uranium acetate and sulphuric acid ferrous sulphate reagents, *Anal. Chem.*, 42, 423–428, 1970.
- Rousse G, Fleischer S and Yama moto A, Two dimensional thin layer chromatographic separation of polar lipid and determination of phospholipids by phosphorus analysis of spots, *Lipids*, 5, 494–496, 1970.



18. Rice E. W, Triglycerides in serum, In standard methods in clinics, Chem. Rodeich, M.P. (Ed), Academic Press, New York, 6, 215–222, 1970.
19. Rai VK, Role of amino acids in plant responses to stresses. *BiolPlant*, 45, 481-487, 2009.
20. Sadasivam S, and Manickam A, Biochemical methods for agricultural science, Wiley eastern Ltd, Madras, 20–21, 56-58, 129–130, 1996.
21. Kapeller and Adler R, Estimation of free amino acids, *Biochem. Z.* 264, 133, 1933.
22. Sakaguchi S, Estimation of arginine, *J. Biochem.*, Tokyo, 5, 133, 1925.
23. Maya KM, John Zachariah T, Krishnamurthy KS, Rema J and Krishnamoorthy B, Fatty acids and leaf amino acids in *Myristica fragrans* and its wild taxa, *Indian J Horti*, 63(3), 316–318, 2006.
24. Hassan, LG, Umar KJ, Dangogo SM. and Ladan MJ, Protein and Amino Acids Composition of African Locust bean (*Parkia biglobosa* L.), *Journal of tropical and Subtropical agro systems*, 2005.

**Source of Support:** Nil, **Conflict of Interest:** None.

