

## Evaluation of Antioxidant and Xanthine Oxidase Inhibitory Potential of Methanolic Extract of *Myristica fragrans* (Mace)

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### Abstract

*Myristica fragrans* is commonly named as nutmeg or mace. It is native to Moluccas of Indonesia. It is used in folk medicine. Phytochemicals present in the plant are responsible for the medicinal properties of the plant. Antioxidants neutralise the free radicals that are produced in our body due to various biochemical reactions. Gout is a clinical condition caused due to accumulation of Uric acid crystals in one or more joints leading to inflammation. Xanthine oxidase inhibitory potential of the extract helps in preventing excess production of uric acid. The extract was found to be rich in phytochemicals such as saponin, alkaloid, terpenoids and flavonoids. The IC<sub>50</sub> for the antioxidant potential of the extract was 280 µg/ml and the IC<sub>50</sub> for the xanthine oxidase inhibitory potential of the extract was 320 µg/ml.

**Keywords:** Antioxidant, Antigout Activity, Innovative Technology, *Myristica Fragrans*, Novel Method, Xanthine Oxidase Inhibitory Potential.

### Introduction

All the plants in the world have medicinal properties. Traditional medicines are affordable and culturally acceptable by a number of people as it has no side effects. It plays a major role in meeting the health care of peoples living all over the world [1]. It is easily available at least cost. WHO has accepted that most of the people living in developing countries use traditional medicine for treating their diseases [2].

*Myristica fragrans* commonly named as nutmeg or mace. It belongs to the family Myristicaceae [3]. It has a seed (nutmeg) which is surrounded by red aril (mace) [4]. It grows to a height of about 5-13m [5]. It is used in folk medicine. It contains 25-30% fixed oil, 5-15% volatile oil, myristic acid, lignin compound. It is native to Moluccas or Spice Island of Indonesia. It adds flavour to food. It is used in perfume. Nutmeg oil is used to treat nervous and digestive systems. It has antioxidant,

antidiarrheal and anti gout activity. For treating mild ringworm, paralysis, chronic rheumatism and sprain concrete oil of nutmeg is used [6]. It lessens the development of atherosclerosis [7]. Nutmeg can increase the dream recall and also vividness of the dreams [8].

Antioxidants neutralise the free radical in our body. They work by terminating the propagation of oxidizing reactions. Nutmeg fruit flesh derived oil acts as a reducing agent and also acts as an anti free radical of DPPH. Argenteane, an antioxidant derived from [9] nutmeg's mace has similar activity as that of Vitamin E. The aqueous extract of nutmeg mace contains lignin which has antioxidant activities. Since the use of synthetic antioxidants is toxic, the need for natural antioxidants derived from plants is increasing. Many aromatic herbs contain antioxidant properties [10]. When compared to other parts of nutmeg, its root has strong antioxidant and anticancer activity [11].

The presence of phytochemicals is responsible for the medicinal properties of plants. They are considered as secondary metabolites. They are synthesised in all parts of the plant body. Phytochemicals are very useful to the pharmaceutical industry [12]. The phytochemicals present in *Myristica fragrans* are carbohydrates, saponin, flavonoids, alkaloids and terpenoids. Terpenoids are used as flavouring compounds and it is a constituent of essential oil [13]. Due to the presence of alkaloids in *Myristica fragrans* it shows antimicrobial activity.

Gout is a form of inflammatory arthritis. It is caused due to elevation of uric acid in the serum that causes accumulation of urate crystals in joints leading to inflammation [14]. As the age increases the incidence of gout also increases. Allopurinol is a standard drug that is used to treat gout. It has been used as an anti-gout drug since 1966 [15]. Allopurinol is a synthetic drug that has many side effects like it causes hypersensitivity and severe cutaneous adverse reaction (SCAR). Xanthine oxidase inhibitory enzymes help in preventing excess production of Uric acid in purine metabolism by inhibiting the enzyme xanthine oxidase [16, 17].

The research is needed to explore the medicinal properties of natural sources. Synthetic anti gout medicines such as colchicine, allopurinol are reported to have various side effects if used for a longer period of time. Anti gout activity of *Myristica fragrans* has not been reported yet. The aim of this study was to evaluate the antioxidant and Xanthine oxidase inhibitory potential of methanolic extract of *Myristica fragrans* mace.

## Materials and Methods

### Chemicals

All chemicals and reagents used for this research work were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA.

### Collection of Plant Material

The *Myristica fragrans* (Mace) was collected from Chennai District, Tamil Nadu, India. The species were identified and authenticated at the Department of Centre for Advanced Study in Botany, University of Madras, Chennai, India. The mace of the plant was shade-dried, cut into small pieces and coarsely powdered. The coarse powder was used for extraction with methanol.

### Preparation of Methanolic Extract of *Myristica Fragrans* (Mace)

1kg of dry powders from *Myristica fragrans* (Mace) were taken in individual aspirator bottles; 3 liters of methanol was used and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled. The extracts were filtered before drying using whatman filter paper no 2 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40°C; the extracts were placed in pre-weighed flasks before drying.

### Phytochemical Screening Test

The phytochemical screening was assessed by the method of Hayat et al., (2017) [18].

### Test For Phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

### Test for Carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

### **Test for Flavonoids**

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

### **Test For Alkaloids**

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

### **Test For Terpenoids**

2 ml of sample along with 2ml of chloroform and 3ml of con. H<sub>2</sub>SO<sub>4</sub> was added. Red color ppt obtained indicates the presence of terpenoids.

### **Test For Proteins**

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

### **Detection of Saponins**

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

### **Test For Steroids**

One ml of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

### **Antioxidant Activity**

#### **Dpph Free Radical Scavenging Activity**

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989) [19]. DPPH solution (1.0 ml) was added to 1.0 ml of

extract at different concentrations (0.1 to 0.5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability of the extract to scavenge the DPPH radical was calculated and expressed in percentage (%).

#### ***In vitro* Xanthine Oxidase Inhibitory Activity Methanolic Extract of Myristica Fragrans (Mace)**

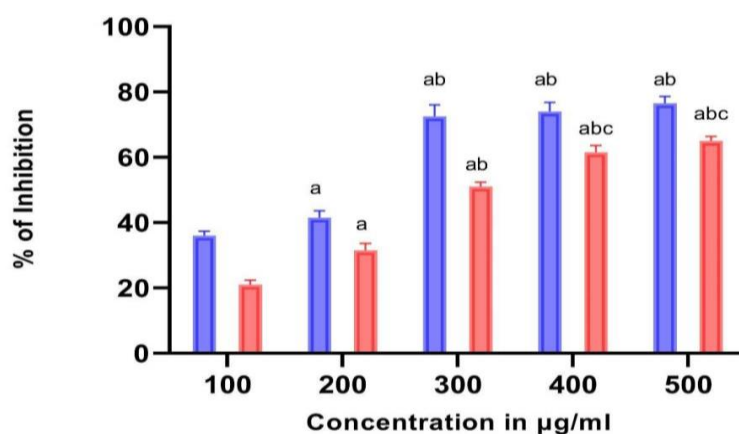
*In vitro* Xanthine oxidase inhibitory of the extract was assessed as per the method of (Nguyen et al, 2004; Umamaheswari et al., 2007). Briefly, the assay mixture consisted of 1 ml of the fraction (0.1 to 0.5g/ml), 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of xanthine oxidase enzyme solution (0.1 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25°C for 15 min, the reaction was initiated by the addition of 2 ml of the substrate solution (150 M xanthine in the same buffer). The assay mixture was incubated at 25°C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer. Allopurinol (0.1 to 0.5mg/ml), a known inhibitor of XO, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25°C. XO activity was expressed as the percentage inhibition of XO in the above assay system.

**Statistical Analysis:** The data were subjected to statistical analysis using two-way analysis of variance (ANOVA) and Tukey's multiple range test to assess the significance of individual variations between the groups. In Tukey's test, significance was considered at the level of  $p < 0.05$ .

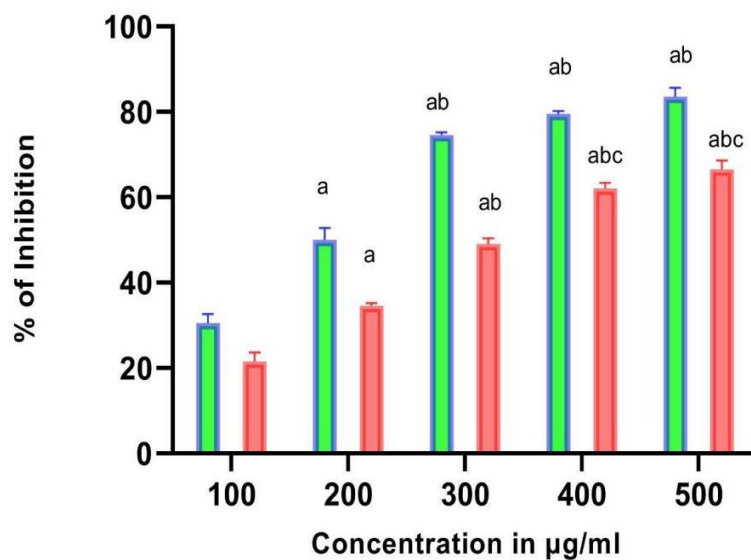
## Results

**Table 1.** Phytochemical Analysis Of Methanolic Extract Of *Myristica Fragrans* ( Mace)

S.No	Phytochemical	<i>Myristica fragrans</i>
1.	Carbohydrates	+
2.	Protein	-
3.	Amino acid	-
4.	Saponin	+
5.	Steroids	-
6.	Flavonoids	++
7.	Alkaloids	++
8.	Terpenoids	++



**Figure 1.** Antioxidant Potential of Methanolic Extract of *Myristica Fragrans* (Mace)-DPPH Assay (blue- ascorbic acid, red- myristica fragrans)



**Figure 2.** Xanthine Oxidase Inhibitory Potential of *Myristica fragrans* (mace). Green - Allopurinol, Red- Myristica Fragrans

## Phytochemical Analysis

From the study, it was evident that the methanolic extract of *Myristica fragrans* (mace) was found to be rich in phytochemicals such as Alkaloids, flavonoids, terpenoids and saponins.(Table 1) . Phytochemicals are the secondary metabolites and determine the plant's medicinal property.

## Antioxidant Analysis

Antioxidant analysis of the methanolic extract of *Myristica fragrans* (mace) was analysed and compared with the standard vitamin- C.  $Ic_{50}$  of the extract was found to be  $280\mu\text{g/ml}$  (Fig 1) Antioxidant potential of the extract was compared with Vitamin C (  $Ic_{50}=250\mu\text{g/ml}$  ).as standard. The antioxidant potential of the extract increased as the concentration increased.

## In vitro Xanthine Oxidase Inhibitory Activity

In vitro Xanthine oxidase inhibitory potential of the extract was assessed and compared with the standard Allopurinol. If the extract exhibits significant inhibition on the enzyme xanthine oxidase it is proportional to its anti gout potential.

The extract exhibited a significant anti gout potential (Fig 2) with an  $Ic_{50}$  of  $320\mu\text{g/ml}$ .

Anti gout potential of the extract increased in a dose dependent manner as compared to the standard- Allopurinol.

## Discussion

Methanolic extract of *Myristica fragrans*(mace) was found to be rich in carbohydrates, saponin, flavonoids, alkaloids and terpenoids. Phytochemicals are present only in plants and the presence of certain phytochemicals determines the antioxidant and medicinal property of the plant extract. In this study, methanolic extract is taken, the choice of solvent also determines the solubility of the phytochemicals and its rich presence in the extract.Further extract has to be prepared with

various solvents to check the presence of other phytochemicals.The presence of phytonutrients like flavonoids and alkaloids indicates that the extract can be a potential antioxidant , antidiabetic and anticancer agent. Further research has to be done to exhibit the medicinal property of the plant.

The extract was analyzed for antioxidant activity by DPPH free radical scavenging assay. Free radicals are molecules having an unpaired electron emerging in oxidative stress. Phenolic compounds have great importance in free radical scavenging activity. In a research by Binawati Ginting et al, the essential oil from the root, bark, and fruit of *Myristica fragrans* showed no strong antioxidant activity. But the essential oil from nutmeg exhibited strong antioxidant potential[20]. In a research by Ilham Maulana et al., the ethyl acetate extract of nutmeg's stem bark has reported a better inhibitory activity[21]. The effect of antioxidants on DPPH free radical scavenging was considered to be due to their hydrogen donating ability. The result obtained in the current study on methanolic extract of *Myristica fragrans* exhibited a significant antioxidant activity.( $Ic_{50}=280\mu\text{g/ml}$  ). Almost significantly equal to the standard vitamin C( $Ic_{50}=250\mu\text{g/ml}$  )

In a previous research done by Rancy Ann Thomas et al., on methanolic extract of *Myristica fragrans* mace as an anti-inflammatory agent, it was reported that the methanolic extract contains terpenoids and concluded that the presence of terpenoids is the major reason for the extract's anti-inflammatory property. Also proved the role of terpenoid in decreasing the inflammation.Similar to this finding , the current study also shows an increased presence of terpenoids in the extract.Gout is an inflammatory disorder and thus reducing the inflammation becomes a major priority in curing the disorder. Thus the extract was analysed for its anti gout potential [12].

A dose dependent Xanthine oxidase inhibitory activity was observed for the extract and drug. In the present study the standard drug allopurinol exhibited greater percentage of inhibition of xanthine oxidase enzyme than the extract in all the given concentrations. Allopurinol is a standard drug used for the treatment of gout. The calculated  $I_{c50}$  value of the extract is  $320\mu\text{g/ml}$ . In a study by Mohammad Fahad Ullah, the % of inhibition of xanthine oxidase depends on the concentration of hydro-methanolic extract of the herb [22]. These results revealed that the standard drug allopurinol has the most potential. Although the extract exhibited less activity than the standard, the extract can be used after purification as it does not show any side effects. Extraction of the principle compound and its purification can increase the activity of herbal drugs. in drug formulation as it does not expect any side-effects. Further research has to be done in *Myristica fragrans* to identify, extract the principle compound to formulate it into new drug formulations in future.

## Reference

- [1] Jaiswal A (2018) Traditional Health Care and Traditional Medicine in India. Archaeology & Anthropology: Open Access. <https://doi.org/10.31031/aaoa.2018.02.000537>
- [2] Kayne SB (2010) Traditional Medicine: A Global Perspective.
- [3] Ross IA (2001) *Myristica fragrans*. Medicinal Plants of the World 333–352.
- [4] Singh DK, Jaiswal P, Kumar P, Singh VK (2009) Biological Effects of *Myristica fragrans*. Annual Review of Biomedical Sciences. <https://doi.org/10.5016/1806-8774.2009v11p21>
- [5] Gupta E (2020) Elucidating the Phytochemical and Pharmacological Potential of *Myristica fragrans* (Nutmeg). Ethnopharmacological Investigation of Indian Spices 52–61.
- [6] J S, Sethi J (2018) *Myristica Fragrans* (MF):

## Conclusion

Gout is caused by hyperuricemia. That is due to excess production of uric acid in the presence of the enzyme xanthine oxidase. Uric acid production can be decreased by inhibiting the enzyme xanthine oxidase. As the synthetic drugs used in the treatment of gout such as colchicine, allopurinol exhibits various severe side effects, more research has to be done on herbal extracts to explore its anti gout potential. In the current study, methanolic extract of *Myristica fragrans* exhibits a significant xanthine oxidase inhibitory potential and thus can reduce the production of uric acid. Further research has to be done to convert the extract into an anti gout drug formulation.

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## Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

Potential Role as an Antioxidant and Anti-Inflammatory Agent. *Journal of Natural & Ayurvedic Medicine*.

<https://doi.org/10.23880/jonam-16000120>

- [7] Liu S, Gao J, He L, Zhao Z, Wang G, Zou J, Zhou L, Wan X, Tang S, Tang C (2021) *Myristica fragrans* promotes ABCA1 expression and cholesterol efflux in THP-1-derived macrophages. *Acta Biochim Biophys Sin* 53:63–71.

[8] Erowid Nutmeg Vault : *Myristica fragrans*: An Exploration of the Narcotic Spice. [https://erowid.org/plants/nutmeg/nutmeg\\_article1.s.html](https://erowid.org/plants/nutmeg/nutmeg_article1.s.html). Accessed 9 Jun 2021.

- [9] Chatterjee S, Niaz Z, Gautam S, Adhikari S, Variyar PS, Sharma A (2007) Antioxidant activity of some phenolic constituents from green pepper (*Piper nigrum* L.) and fresh nutmeg mace (*Myristica fragrans*). *Food Chemistry* 101:515–523.

[10] Thakur M, Paul A, Chawla S (2014) Qualitative

phytochemical screening, total phenolic content and antioxidant activity in methanolic extracts of *Myristica fragrans* Houtt. (Mace). *Food Science Research Journal* 5:135–138.

[11]Ealla KKR, Veeraraghavan VP, Ravula NR, Durga CS, Ramani P, Sahu V, Poola PK, Patil S, Panta P (2022) Silk Hydrogel for Tissue Engineering: A Review. *J Contemp Dent Pract* 23:467–477

[12]Patil S, Sujatha G, Varadarajan S, Priya VV (2022) A bibliometric analysis of the published literature related to toothbrush as a source of DNA. *World J Dent* 13:S87–S95

[13]Caputi L, Aprea E (2011) Use of terpenoids as natural flavouring compounds in food industry. *Recent Pat Food Nutr Agric* 3:9–16.

[14]Kapoor B, Kaur G, Gupta M, Gupta R (2017) Indian Medicinal Plants Useful In Treatment of Gout: A Review For Current Status And Future Prospective. *Asian Journal of Pharmaceutical and Clinical Research* 10:407.

[15]Yasuda T, Yoshida T, Goda AE, Horinaka M, Yano K, Shiraishi T, Wakada M, Mizutani Y, Miki T, Sakai T (2008) Anti-gout agent allopurinol exerts cytotoxicity to human hormone-refractory prostate cancer cells in combination with tumor necrosis factor-related apoptosis-inducing ligand. *Mol Cancer Res* 6:1852–1860.

[16]Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, Zhang Q (2019) Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on

melanoma cells (B16). *Artif Cells Nanomed Biotechnol* 47:3297–3305.

[17]Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui C (2019) 6-shogaol, a active constituents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating Nrf2 signaling in human epidermal keratinocytes (HaCaT cells). *J Photochem Photobiol B* 197:111518.

[18] Ganapathy D, (2021) Awareness of hazards caused by long-term usage of polyethylene terephthalate (PET) bottles. *Int J Dent Oral Sci* 2976–2980

[19]Yoshida T, Mori K, Hatano T, Okumura T, Uehara I, Komagoe K, Fujita Y, Okuda T (1989) Studies on inhibition mechanism of autoxidation by tannins and flavonoids. V. Radical-scavenging effects of tannins and related polyphenols on 1,1-diphenyl-2-picrylhydrazyl radical. *Chemical and Pharmaceutical Bulletin* 37:1919–1921.

[20]Ginting B, Maira R, . M, Helwati H, Desiyana LS, Mujahid R (2018) Isolation Of Essential Oil of Nutmeg (*Myristica Fragrans* Houtt) and Antioxidant Activity Test With Dpph. *Jurnal Natural* 18:11–17.

[21]Priya DV, (2020) Knowledge and awareness on HIV/AIDS among college students in A university hospital setting. *Int J Dent Oral Sci* 1182–1186

[22]Karthik EVG, Priya V (2021) Gayathri. R, Dhanraj Ganapathy. Health Benefits Of *Annona Muricata*-A Review. *Int J Dentistry Oral Sci* 8:2965–2967.