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Phytochemical screening, antimicrobial and antioxidant activities of *Myristica fatua* Houtt. var. *magnifica* (Beddome) Sinclair

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The methanolic and aqueous seed extracts of *Myristica fatua* var. *magifica* were obtained by soxhlet method and used for biochemical studies including antimicrobial and antioxidant activities using standard protocols. Methanolic extract showed the presence of phytochemical constituents like carbohydrates, proteins, alkaloids, tannins, phenolics and resins whereas the aqueous extract showed the absence of phenolics, tannin and resin. Antimicrobial activities were done against five bacterial species viz., *Staphylococcus aureus*, *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Bacillus subtilis* by agar well diffusion method. Out of two extracts, methanol extract were found to be more active against all the microorganisms than the aqueous extract. Antioxidant activity was determined by DPPH method which showed antioxidant potential of methanolic extract compared with the standard ascorbic acid.

Key words: Phytochemical sceening, antimicrobial activity, antioxidant activity, *Myristica fatua* var. *magnifica*.

INTRODUCTION

India is surrounded by water on its three sides, the west coast consist of the Western Ghats extending from Tapti in Gujarat to Kanniyakumari in Tamil Nadu including Maharashtra, Goa, Karnataka and Kerala. The Western Ghats are stretch of hill ranges which are known to be rich in flora and fauna, comprising about 17000 species of flowering plants are estimated in India of which, 4500 species are found in the Western Ghats. Myristicaceae is one of the important families of flowering plants comprising 19 genera and 400 species widely distributed, among which the genus *Myristica* comprises 80 species (Mabberley DJ, 1987). There are five species of Myristicaceae belonging to three genera excluding the cultivated *M. fragrans* in the Western Ghats region of Karnataka. They are *G. farquhariana, Knema attenuata, M. fatua* var. *magnifica, M. malabarica* and *M. dactyloides*. *M. fatua* var. *magnifica* is dominant species found in *Myristica* swamp forest, which is the habitat of this species. Due to anthropological activity and conversion of this habitat to cash crop plantations and teak forests, the fresh water swamp now dwindled to small fractions in Karnataka (Rama Bhat P. and Kaveriappa KM, 2009).

M. fatua Houtt. var. *magnifica* (Beddome) Sinclair is a lofty tree up to 30 m tall, trunk when young furnished with large aerial roots from the trunk. Leaves oblong to elliptic, rounded at base, acute to acuminate at apex, silvery beneath (Plate 1a). Flowers in clusters, small, deciduous, ovoid, up to 2cm long densely rusty tomentose. Male flowering is 10-20 flowered cymes or umbels of 2-3cm long stamens 10. Female flowers are 4-6 flowered cymes or umbels of 1-2cm long (Rama Bhat P. and Kaveriappa KM, 1996. Fruits oblong, up to 10 cm long densely tomentose. Seeds subcylindric or ellipsoid, up to 5cm long, aril deeply much laciniate, orange-red (Plate 1b). Flowering and fruiting from March-October. It is endemic, rare and threatened. It is commonly known as Choora pannu, as Dodda yele Ramapatre in Kannada, Kotthapanu or Kothapayin or Churapayin in Malayalam. It is distributed in as remnant patches in Southern Western Ghats: Kerala- Kollam, Kozhikode, Thiruvananthapuram; Karnataka-Uttara Kannada, Shimoga; Tamil Nadu- Tirunelveli (Rama Bhat P. and Kaveriappa KM, 2009).

India has an ancient heritage of traditional medicine. The *Materia Medica* of India provides a great deal of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicines are based on various system including Ayurveda, Siddha, Unani and Homeopathy. Plants are one of the most important sources of medicine. Today the large numbers of drugs in use are derived from plants (Padmaa M, 2009). The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. The important advantages claimed


Plate 1a: The leaves of *M.fatua* var. *magnifica*, 1b: Dehisced fruits with arilled seed

for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability.

The chemical compounds that occur naturally in plants and responsible for colour and organoleptic properties, such as deep purple of blue berries and smell of garlic are called photochemicals. These phytochemicals may have biological significance but are not established as essential nutrients. Scientists estimates that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome. These phytochemicals are abundant in fruits, vegetables and herbs (Sunil H, *et al*., 2012).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, are of great significance to therapeutic treatments. Extracts of plants were used for the treatment of various diseases and this forms the basis for all Indian system of medicines. However this area is not much developed when compared to modern system of medicines, mainly because of the lack of scientific documentation in this field (Hemraj V. and Anil J., 2012). Recent investigations revealed that plant origin antioxidants have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurogenerative diseases, cardiovascular diseases, aging, gastrointestinal diseases. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effect; while relatively plant based medicine confer fewer side effects than the synthetic drugs in some instances (Dev KU, *et al*,.2012).

Myristica fatua var. *magnifica* belongs to the family *Myristicaceae* (nutmeg family). The other species of this family contains antimicrobial and antioxidant activity. There is one report on the use of aril covering the seed of this plant used as dyeing in Dakshina Kannada; the wood has no other uses [8]. The above properties may be present in *M. fatua* var. *magnifica* seeds and arils. So a detailed study is required for analysis. But exploitation of plant should be narrowed as the species is listed in the RED List of Plants which needs special care of conservation strategy. Conservation of habitat as well as fresh water ecosystem – the *Myristica* swamp needs first rank of attention in the biodiversity conservation acts. The seeds are of recalcitrant type, looses viability rapidly.

Nutmeg (*M. fragrans)* contains many plant-derived chemical compounds that are known to have been anti-oxidant, disease preventing, and health promoting properties. The active principles in nutmeg have many therapeutic applications in many traditional medicines as anti-fungal, anti-depressant, aphrodisiac, digestive, and carminative functions. Alcoholic extract of nutmeg have antibacterial activity against *Micrococcus pyrogenes* var. *aureus.* Aqueous decoctions are toxic to cockroaches. Myristicin is used as an additive to pyrethrum to enhance its toxicity against houseflies. The leaf essential oils have weedicidal properties. It is also used for making soaps, dentifrices, chewing gums and tobacco. Nutmeg pericarp is used in pickles and jellies. Nutmeg oil contains *eugenol*, which has been used in dentistry for toothache relief. The oil is also used as a local massage to reduce muscular pain and rheumatic pain of joints. Freshly prepared decoction with honey has been used to relief of nausea, gastritis, and indigestion ailments (Anonymous, 1962). On the other hand *M. malabarica* (wild nutmeg) seed used in external application for indolent ulcers, crude fat from seeds analgesic and used in rheumatism and gangrene. The yellowish maize is used as an adulterant for true mace. The seed and aril extracts possess antifungal and antibacterial activity.

Based on the above observations a research study has been undertaken with the following objectives:

- To analyse the phytochemical constituents in the aqueous and methanolic extract of the seed of *Myristica fatua* var. *magnifica.*
- To evaluate antimicrobial activity of methanolic and aqueous extract on selected bacterial and fungal strains.
- To study the antioxidant activity of aqueous and methanolic extracts of the seed of *M. fatua* var. *magnifica.*

MATERIALS AND METHODS

Collection of sample: *Myristica fatua* var *magnifica* seeds were collected from Kathal kane evergreen swamp forests of Shimoga, near Jog falls, Karnataka during September – October 2015. The seeds and leaves were allowed to shade dry for a week. These were then kept in hot air oven at 60°C for 24-48 hours until it was dried completely. These were then coarsely powdered and stored in a closed container for further use.

Test organisms: *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi* were used as test organisms from the P.G Department of Biotechnology, Alva's College and microscopic examination was done for the confirmation and were maintained in slants.

Preparation of aqueous and methanolic extract: The coarse powder of the seed (40 g) was extracted by soxhlation process. The mass thus obtained was extracted for 1hour with 150 ml of distilled water. This was followed by the distillation process. The extract thus obtained was dried in water bath at 40-60^{0}C for a week. Similarly methanolic extract is obtained using methanol. The dried extracts thus obtained were used for assessment of antimicrobial and antioxidant activities.

Phytochemical screening and quantification of the plant extract: Qualitative tests for carbohydrate, proteins, alkaloids, phenolics, tannins and resins were performed using standard protocols (Sadasivam S. and Manickam A, 2008).

Antibacterial screening of plant extract: A loop full of culture was inoculated into nutrient broth and incubated at 37^{0}C for 24 hours to obtain the bacterial cultures.

Antibacterial activity test by agar well diffusion method: The agar well diffusion method was employed to assay the plant materials for antimicrobial activity. Petri dishes were plated with Muller Hinton Agar media and allowed to solidify for 30 minutes. The test organisms were then spread on the surface of the media using sterile ear buds. Cork borer (4mm) was used to bore wells in media. The methanolic extract of different concentrations viz., 25, 75, 100, 300, 500 and 1000 µg/ml were dispensed into the wells using a micropipette. A negative control of methanol and a positive control of streptomycin were kept and the extract was allowed to diffuse for 1 hour at room temperature. For aqueous extract, distilled water serves as control. Then the plates were incubated at 37^{0}C for 24 hours. Zone of inhibitions were measured.

Antifungal activity by poison drop method: Standard extracts were swabbed on to the Potato Dextrose Agar (PDA) plates. The seven day old fungal cultures disk of 5 mm diameter was taken and inoculated at the centre of Petri plates containing plant extracts in aseptic condition. In the Potato Dextrose Agar medium, without the plant extracts served as control. All the PDA plates were incubated at 28±2^{0}C and radial growth of colony was measured after 7 days of incubation. Each test was performed in duplicates for both methanolic and aqueous extract.

Antioxidant activity by DPPH method (Dev KU, *et al*., 2012)**:** The free radical scavenging activity of the extract was measured *in vitro* by 2,2'-diphenyl-1-picrylhydrazyl (DPPH). The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and stored at 20^{0}C until required. A 3 ml aliquot of this solution with 100 µl of the sample of various concentration (20, 60, 100, 500 and 1000 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 minutes at room temperature. Then the absorbance was taken at 570 nm. The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Control absorbance } (A_0) - \text{Sample absorbance } (A)}{\text{Control absorbance}} \times 100$$

RESULTS AND DISCUSSION

Extraction and qualitative analysis of phytochemicals: The per cent yield of methanolic and aqueous seed extracts of *M. fatua* var *magnifica* were 50.0 and 12.5 respectively. The aqueous extract showed positive results for carbohydrates, proteins and alkaloids whereas the methanolic extract showed positive results for carbohydrates, proteins, alkaloids, phenolic compounds, tannins and resins.(Table 1). In the present study, the greater yield of the extract was found in methanol as compared to the aqueous extract. One of the study reported by Deepa PR, *et al.* (2015) the methanolic extract produced the greater yield as compared to the aqueous extract of fruit, bark and leaf of

Samadera indica. There are some earlier reports which support the present work (Akhila Zainab, *et al*., 2013; Ashuthosh Yende, *et al*., 2013; Prajna PS and Rama Bhat P, 2015).

Table 1: Phytochemical analysis for methanol and aqueous extract of the plant *Myristica fatua* var. *magnifica*

Phytochemicals	Aqueous extract	Methanol extract
Carbohydrates	+	+
Proteins	+	+
Alkaloids	+	+
Tannins	-	+
Phenolics	-	+
Resins	-	+

+ indicates present, - indicates absent

The phytochemical and pharmacological studies of seed extracts of *Myristica fatua* var. *magnifica* were carried out by the standard techniques. The qualitative test showed the presence of carbohydrates, proteins, alkaloids, tannins, phenolics and resins. The quantitative estimation showed the presence of 0.06 mg/g of carbohydrates, 0.112 mg/g of proteins, 0.086 mg/g of phenolics and 0.068 mg/g of tannins. Similarly, Thomas AR and Krishnakumari S (2015) anlysed the dried seeds of *Myristica fragrans* using different solvent extracts such as methanol, ethanol, ethyl acetate, chloroform, petroleum ether, acetone and aqueous and revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, saponins, flavonoids, tannins, steroids. The ethanolic, methanolic and aqueous extract revealed the maximum presence of phytoconstituents where as chloroform, petroleum ether and ethyl acetate extracts showed minimal amounts of phytoconstituents. Rama Bhat P. and Kaveriappa KM (1998) analysed the chemical composition of kernel and mace (aril) of *M. fatua* var. *magnifica* using raw materials and reported that the non volatile oil content was about half when compared to nutmeg but the starch, protein and ash contents were higher than that of nutmeg.

Determination of antibacterial activity by agar well diffusion method: The bacterial cultures showed no levels of sensitivity towards different concentrations of aqueous extracts. The bacterial cultures showed varied levels of sensitivity towards different concentrations of methanolic extracts. *Staphylococcus aureus, Klebsiella pneumoniae and Salmonella typhi* showed high sensitivity for the methanolic plant extract (Table 2). In the present study, methanolic extract was found to be more resistant to all the organisms than the aqueous. The highest zone of inhibition was found in the concentration of 1000 µg/ml for *Klebsiella pneumoniae* (21.5 mm), *Staphylococcus aureus* (20.0 mm), *Salmonella typhi* (19.5 mm) in methanolic extract. In anothere study by Ameen SJ (2012), the 100% concentration, the aqueous and ethanolic extracts of *Myristica fragrans* were effective against *E. coli* with inhibition zones of 16 mm and 19 mm respectively while in 75% concentration, the inhibition zones were 14 mm and 19 mm respectively. The 100% concentration, the aqueous and methanolic extract of nutmeg was effective against *Staphylococcus aureus* with inhibition zones of 12 mm and 13 mm respectively. On the other hand, Kulandhaivel M and Palaniswamy M (2012) found that the hydroethanolic extracts of *M. fragrans* and *Camellia sinensis* exhibited antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*.

Table 2: Antibacterial activity for the methanolic seed extract of *M. fatua* var. *magnifica* at different concentrations

Test organisms	**Zone of inhibition (mm) for different concentrations of methanolic extract of seed**							
	25µl	**75µl**	**100µl**	**300µl**	**500µl**	**1000µl**	**Control 1**	**Control 2**
Staphylococcus aureus	13.0	11.0	13.5	15.0	17.5	20.0	31.0	9.5
Klebsiella pnuemoniae	12.0	13.0	13.5	15.0	17	21.5	30.5	9.5
Escherichia coli	9.0	9.0	10.0	12.0	15.5	19.5	30.5	9.0
Salmonella typhi	11.5	11.0	13.0	14.5	17.5	20.0	30.5	9.5
Bacillus subtilis	11.0	10.5	11.0	13.0	15.0	19.0	31.0	9.0

Control 1 = Streptomycin, Control 2= methanol

Antifungal activity by poison drop method: The antifungal activity of the various concentrations of methanolic extracts of *M. fatua* var. *magnifica* was carried out against the various strains of fungi such as *Aspergillus fumigatus, A. niger* and *Candida albicians.* The methanolic extract showed activity against *A. niger* and *Candida albicans* but no clear zone of inhibition was observed against *A. fumigatus* (Table 3). Hence the extract is resistant to *A. fumigatus. Candida albicans and A. niger exhibited* the highest zone of inhibition at the concentration 500 µg/ml. On the other hand, Gupta AD, *et al*. (2013) observed that acetone, aqueous and butanol extracts of *M. fragrans* against the bacterial strains such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas putida* and *P. aeruginosa* showed activity. The antifungal activity was also found highest in acetone extract with *Aspergillus niger* (14.4± 0.37mm). Similar results have

been reported by Joseph J and George M (2014) in the methanolic and ethanolic extracts of *M. fragrans* pericarp which showed remarkable activities against *Staphylococcus aureus* and *Salmonella typhi* respectively as compared to the hexane extracts. In one of the earlier studies different solvent extracts were tested against *S. aureus* and *E. coli* which showed effective zone of inhibition ranging from 20 mm to 8 mm with MIC 512ug/ml- 128ug/ml (Surbhi Kaushik and Padma Singh, 2012).

Table 3: Table: *In vitro* antifungal activity by Poison Drop method

Organisms	**Zone of inhibition (mm)**	
	Control	Methanolic extract – 500 µg/ml
Candida albicians	4.5	1.8
Aspergillus fumigatus	6.0	8.0
Aspergillus niger	8.0	5.5

***In vitro* antioxidant activity by DPPH method:** The scavenging effects of the methanolic extract on DPPH radical were higher as compared to aqueous extract. Though the antioxidant potential of the methanolic extract was found to be lower than the ascorbic acid, the study revealed that the methanolic extract have prominent antioxidant activity (Fig. 1); the presence of phenolic compounds are mainly found in this extract and could be attributable to the observed high antiradical properties of this extract. The methanolic extract of *M. fatua* var. *magnifica* is showing antioxidant activity which is comparable to standard ascorbic acid. Among the different concentration of the extract, 500 µg/ml showed 42.8% inhibition. The study revealed that the methanolic extract have the prominent antioxidant activity; the presence of phenolic compounds are mainly found in this extract and could be attributable to the observed high antiradical properties of this extract. Similar study was conducted by Manjunatha BK, *et al*. (2012), whereby the *in vitro* antioxidant activity of *M. malabarica* by DPPH method. The methanolic extract exhibited good I_C values at 0.02 mg/ml in DPPH radical scavenging assay, 0.107 mg/ml in scavenging of Hydrogen peroxide assay, 1.6 µg/ml in ABTS radical cation decolourization assay and 0.5 mg/ml in nitric oxide scavenging assay respectively.

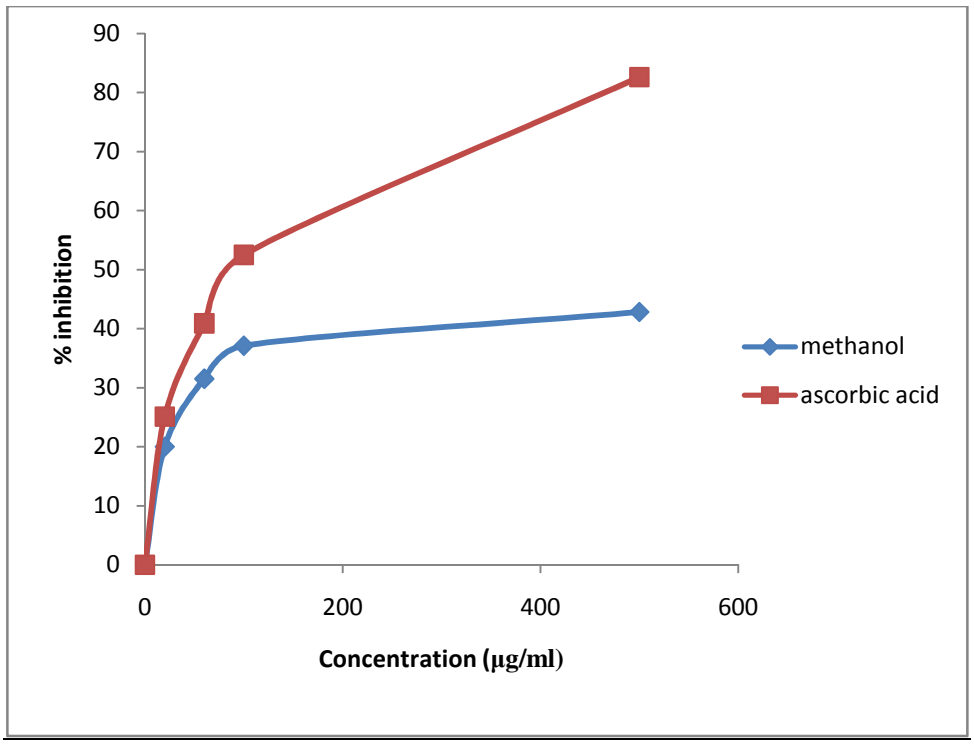

Fig. 1: DPPH radical scavenging activity of methanolic extract of *M. fatua* var. *magnifica* and standard

One of the recent work on the free radical scavenging activity of purified natural lignin dimmer isolated from *M. fragrans* by DPPH method, showed that 100,10,1 and 0.1µg/ ml of purified lignan had 76.7%, 65%, 28% and 8% scavenging activity respectively while the same concentrations of partial purified lignan had 44.3%, 18.5%, 11% and 0% scavenging activity respectively (Al-Jumaily EF, *et al*., 2015). This further supports the present study that similar effect could be exhibited by this species with other species of the genus *Myristica.* Similar results were observed in methanolic extracts of different plant species. The total antioxidant activity of the bark extracts of *Pajanelia longifolia* was found to be in the range of 250 to 2600 mM Fe (II)/g by FRAP assay. Among the four extracts used, 70% methanol extract showed the highest antioxidant activity (Akhila Zainab, *et al.,* 2013). The present work supports the efficacy of *M. fatua* var. *magnifica* towards its antimicrobial activity and antioxidant activity compared with nutmeg and wild nutmeg. As the plant is considered as rare and threatened, utilization of plant under control and preference should be given to conservation of gene pool.

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