International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 3; Issue 1; January 2018; Page No. 52-56

Evaluation of bioactive compounds in different extracts of *Myristica fragrans* and their antioxidant potential

[1] Abhilasha Singh, [2] Ritu Thakur Bais
[1] Department of Microbiology, Barkatullah University, Bhopal, Madhya Pradesh, India
[2] Department of Botany, Govt. M.L.B. Girls (Auto.) P.G. College, Bhopal, Madhya Pradesh, India

Abstract
In the present study methanolic, aqueous and hydroalcoholic extracts of *Myristica fragrans* were evaluated using various *in vitro* antioxidant assays including nitric oxide scavenging capacity, total antioxidant activity and measurement of reducing power through hydroxyl reducing antioxidant capacity. The most prominent antioxidant activity was observed with the methanolic extract in the NO scavenging assay with an IC50 value of 473.40±0.69µg/ml as opposed to that of standard ascorbic acid and other extracts. The maximum NO scavenging was 79.1%. In total antioxidant capacity method, methanolic and aqueous extracts showed the highest activity. Methanol extract along with hydroalcohol were also found to exhibit significant and paramount hydroxyl reducing capacity respectively in a dose dependent manner.

Keywords: total antioxidant, NO scavenging, H_2O_2 assay, *M. fragrans*

Introduction

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as by product of biological reaction or from exogenous factors [1]. However, these ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects such as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neuro-degenerative diseases [2, 3]. A potent broad spectrum scavenger of these species may serve as a possible preventive intervention for free radical mediated cellular damage and diseases [4]. Antioxidant based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer's disease and cancer have appeared during last three decades. Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exerted an antioxidant action [5, 6]. There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer [7]. There is currently immense interest in natural antioxidants and their role in human health and nutrition. Considerable amount of data have been generated on antioxidant properties of food plants around the globe [8]. However, traditionally used medicinal plants await such screening. On the other hand, the medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability.

Nutmeg (*Myristica fragrans* (H.) seed is widely used as a spice, is a tropical, dioeciously evergreen tree native to the Moluccas or Spice Island of Indonesia. Nutmeg has a characteristic pleasant fragrance and is slightly warm taste. It is used to flavour many kinds of baked goods, confections, puddings, meats, sausages, saucers, vegetables, and beverages [9]. It is also used as components of curry powder, teas and soft drinks, or mixed in milk and alcohol. The main reason to choose nutmeg for this study is that, in spite of traditional use in numerous medical conditions, nutmeg has not been comprehensively evaluated for their antioxidant and antimicrobial potential, which could be contributed by the variety of active phytochemicals including vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans and phenolics, etc. These compounds render their effects via different mechanisms such as radical scavenging, metal chelation, and inhibition of lipid peroxidation and quenching of singlet oxygen to act as antioxidants.

Methodology

Total antioxidant activity

Total antioxidant activity was estimated by phosphomolybdenum assay [10]. 1ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water. The extract of *M. fragrans* in different concentration ranging from 200 µg to 1000 µg was added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95ºC for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard.

Nitric oxide scavenging activity

The procedure was based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline (PBS) was mixed with different concentrations of all extracts dissolved in methanol and incubated at room temperature for 150 minutes. The same reaction mixture without the extracts but the equivalent amount of methanol served as the control. After the incubation period, 0.5 ml of Greiss reagent [1% sulfanilamide, 2% H3PO4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride] was added. The absorbance of the chromophore formed was read at 546 nm using a spectrophotometer [11].

Hydrogen peroxide reducing power

This concept has been exploited for determination of H_2O_2 in the samples. In this assay, after adding ferrous ion, the scavenger is added, followed by hydrogen peroxide reducing power known amount of H_2O_2 for few minutes. If the scavenger is capable enough to scavenge the H_2O_2 added in the sample, no ferrous to ferric conversion would occur and this is detected by addition of 1,10-phenanthroline to yields a red-orange complex. Conversely, if the scavenger is unable to scavenge H_2O_2, then H_2O_2 converts all the ferrous ion to ferric which is unable to form coloured complex with 1,10-phenanthroline. Hence, the generation of red-orange ferrous-triphenathroline complex will be directly proportional with the ability and concentration of the scavenger. Accordingly, the calculation of the ability of compounds having hydrogen peroxide scavenging activity was calculated using following formula.

$$\% \ H_2O_2 \text{ scavenging activity} = \frac{\text{A test X 100}}{\text{A blank}}$$

where A blank is the absorbance of solution containing only ferrous ammonium sulphate and 1,10-phenanthroline and A test is the absorbance of the solution containing ferrous ammonium sulphate, hydrogen peroxide along with test compound having expected hydrogen peroxide scavenging activity and 1,10-phenanthroline.

Statistical Analysis: The data acquired from in vitro analysis were communicated as mean standard error (±S.E.M.).

Results and Discussion

Hydroxyl scavenging assay

M. fragrans (H.) extracts showed *in vitro* antioxidant activity in hydroxyl radical scavenging activity in dose dependant manner. At 100 µg/ml concentration of methanol, water and hydroalcohol extracts, it showed 82.46%, 62.51% and 76.39 % inhibition in hydroxy radical respectively (Table 1). IC 50 values of methanol, water and hydroalcohol extract are 39.72±0.31, 58.67±0.33 and 34.99±0.53µg/ml in hydroxyl radical assay respectively (Table 2). Figure 1 and Table 1 show the antioxidant activity of *M. fragrans* (H.) extracts against hydroxyl radical. Methanol extract was observed to highest scavenge activity for hydroxyl radical. In some previous studies it is reported that the antioxidant activity was concomitant with the reducing power [12]. The reducing power of the compounds isolated from nutmeg was investigated to evaluate their antioxidant potentials. Al-Jumaily *et al.*, 2015 [13] reported antioxidant and reactive oxygen species induction using purified natural lignan dimmer isolated from *M. fragrans* (H.) seed. Superoxide radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to tissue damage and various diseases [14]. The effectiveness of the above mentioned plant extracts to quench hydroxyl radicals seems to insinuate that they are good scavengers of oxygen species. It has already been discussed that the potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution [15].

Table 1: Hydroxyl scavenging activity of methanol, water and hydroalcohol extract of *M. fragrans* (H.).

Concentration (µg/ml)	Methanol	Aqueous	Hydroalco.
0	0	0	0
20	9.58	35.06	39.45
40	16.71	33.69	46.3
60	20.95	35.89	55.61
80	36.98	38.9	52.87
100	59.72	40.82	67.67

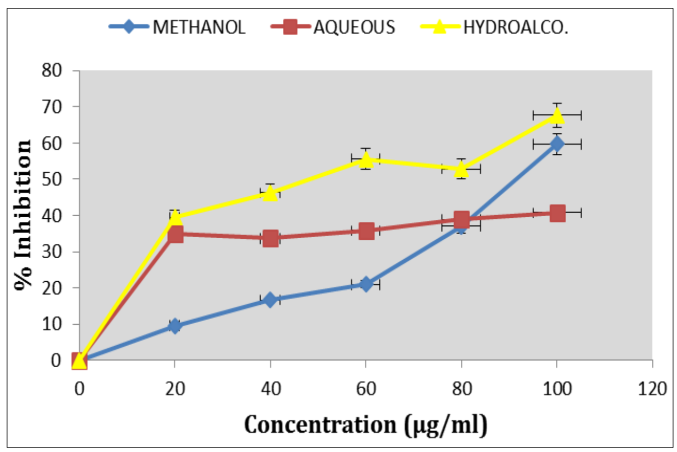

Fig 1: Hydroxyl scavenging activity of methanol, water and hydroalcohol extract of *M. fragrans* (H.).

Table 2: IC50 values of different extracts of *M. fragrans* (H.) and *A. indica (A.)* for Hydroxyl scavenging

	IC50	Values for hydroxyl	Redicals
Plants	Methanol	Aqueous	Hydroalcohol
Myristica fragrans (H.)	39.72±0.31	58.67±0.33	34.99±0.53

Total Antioxidant Assay

Antioxidant activities of essential oils from aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them [16]. In this study, the antioxidant activities related to the contents of *M. fragrans*

(H.) were determined. The results are summarized in Figure 1 &Table 3. It was reported that the different extracts showed very different antioxidant capacities. Stronger activity is indicated by aqueous extract. Generally, yield and antioxidant activities of extracts are relying on nature of the extracting solvent due to the presence of distinct antioxidant compounds with different chemical characteristics and polarities of polar solvent. In determining the antioxidant capacity, aqueous extract appeared to have the highest TAA values. This showed that *M. fragrans* possess greatest reducing property and radical scavenging ability compared to other extracts of nutmeg. The antioxidant property is highly relied on their redox properties and chemical structure [17]. According to Shan *et al*. (2005) [18] the high antioxidant activity in nutmeg seed was contributed by caffic acid and catechin. These two groups are good antioxidants due to their catechol structure which is able to donate phenolic hydrogen or electrons to acceptors such as reactive oxygen species or lipid peroxyl groups easily. It has been reported that the total phenolic content and anti-oxidant potential of a product has a significant and positive correlation.

Chakraborty *et al.*, 2015 [19] reported bioactivity of *M. fragrans* (H.) *in* methanol extract. In present work methanol extract showed moderate antioxidant activity. The reducing power of methanol extract indicates presence of some compounds in *M. fragrans* (H.) extracts which can donate electron and could react with free radicals to convert them into more stable products and to terminate radical chain reactions. In another study, it was reported that methanolic extract of nutmeg seed showed good antioxidant activity by methods of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) due to high content of tannin, flavonoid and terpenoids [20].

Table 3: Total antioxidant activity of *M. fragrans* (H.) plant extracted with different solvents

Concentration (µg/ml)	Methanol	Aqueous	Hydroalco.
0	0	0	0
200	0.13	0.515	0.134
400	0.135	0.832	0.289
600	0.147	1.082	0.7
800	0.405	1.251	0.893
1000	0.502	1.36	1.259

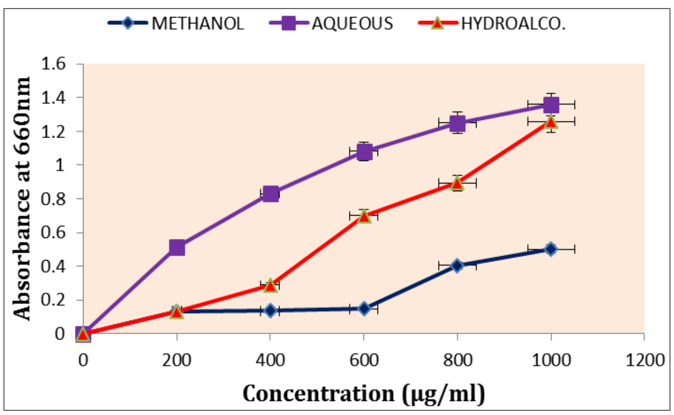

Fig 2: Total antioxidant activity of *M. fragrans* (H.) plant extracted with different solvents

Table 4: IC50 values of different extracts of *M. fragrans* (H.) and *A. indica (A.)* for TAA

	IC5	Values for TAA	
Plants	**Methanol**	**Aqueous**	**Hydroalcohol**
Myristica fragrans (H.)	383.24±1.38	385.35±3.12	1000.38±42.64

Nitric Oxide scavenging assay

The results of methanol, water and hydroalcohol extracts are shown in the Figure 3 & Table 5. Methanol extract showed a significant decrease scavenging activity when compare to water and hydroalcohol extract. Nitric oxide is an unstable free radical involved in many biological processes which is associated with several diseases. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be toxic and inhibition of over production is an important goal [21]. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Based on the results obtained from present investigation revealed that the nitric oxide radical scavenging activity of *M. fragrans* (H.) extracts showed a significant increase in scavenging of nitric oxide. Results are coherent with the work of Bor *et al*. (2006) [22] who showed that extract of *Asparagus officinalis L* exhibited good nitric oxide scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The reduction of the nitrite concentration might be attributed to α-pinene, β-pinene, myrcene, 1,8-cineole, carvacrol, terpinen-4-ol, eugenol and isoeugenol contain in the extracts [16].

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases [23]. NO is generated in biological tissues by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citralline with the formation of NO via a five electron oxidative reaction [24]. These compounds are responsible for altering the structural and functional behavior of many cellular components. Incubation of solutions of sodium nitroprusside in PBS at 25˚c for 2 h resulted in linear time dependent nitrite production, which is reduced by the tested methanolic extracts of the plants. The results of NO scavenging activity of the selected plant extracts are shown as percent of NO scavenging in figure 3. Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with superoxides, such as NO_2, N_2O_4, N_3O_4, NO_3 and NO_2 are very reactive.

Compounds are responsible for altering the structural and functional behavior of many cellular components. Incubation of solutions of sodium nitroprusside in phosphate buffer saline at 25º C for 2 h resulted in linear time-dependent nitrite production, which is reduced by the tested methanolic, water and hydroalcoholic extracts of *M. fragrans* (H.) seeds. This may be due to the antioxidant principles in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. It is to be noted that methanolic extract a greater inhibition comparative to other extracts. The maximum NO scavenging of methanolic, water and hydroalcoholic extracts were 79.1%, 61.5% and 72.1% with IC50 values 473.40±0.69 µg/ml, 571.25±7.30µg/ml and 784.50±0.31µg/ml respectively (Table 6).

Table 5: Nitric oxide scavenging assay of *M. fragrans* (H.)

Concentration (µg/ml)	Standard	Methanol	Aqueous	Hydroalco.
0	0	0	0	0
200	23	45.01	43.7	6.64
400	38	59.4	55.2	26.3
600	45	57.8	58	37.4
800	57	69.4	59.8	41.4
1000	64	79.1	61.5	72.1

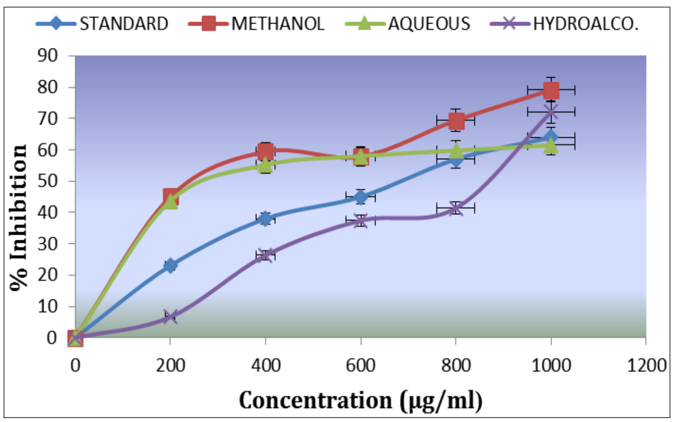

Fig 3: Nitric oxide scavenging assay of *M. fragrans* (H.)

Table 6: IC50 values of different extracts of *M. fragrans* (H.) and *A. indica (A.)* for nitric oxide assay

	IC50	Values for NO	
Plants	Methanol	Aqueous	Hydroalcohol
Myristica fragrans (H.)	473.40±0.69	571.25±7.30	784.50±0.31

Conclusion

The results of the present investigation revealed that the solvent system is highly responsible for the extraction of different phytochemicals. This study concluded that the methanolic and hydroalcoholic extracts of *M. fragrans* (H.) contain high amount of phenol and flavonoid. Based on the various *in vitro* assays it could be concluded that the methanolic and hydroalcoholic plant extracts possess significant antioxidant activity and provide a scientific rationale for the traditional use of this plant. The observations found in the present study support this view that the medicinal plants hold a valuable source of potential antioxidants for the discovery of natural-product pharmaceuticals and to be used as preventive agents in the pathogenesis of various diseases. Further works on identification and isolation of active constituents in the extracts may be exploited by *in vivo* study to determine the underlying mechanism of the overall antioxidant activity.

References

1. Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. Journal of Food Science. 1993; 58(6):1407-1410.
2. Gyamfi MA, Yonamine M, Aniya Y. Free radical scavenging action of medicinal herbs from Ghana *Thonningia sanguinea* on experimentally induced liver injuries. Gen. Pharmacol. 2002; 32:661-667.
3. Noda Y, Anzai-Kmori A, Kohono M, Shimnei M, Packer L. Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR spectromoter system. Biochem. Mol. Biol. Inter. 1997; 42:35-44.
4. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol. 1998; 62:183-193.
5. Al-Jumaily EF, Al-Shanon AA, Al-Barzanchi SI. Antioxidant and reactive oxygen species induction using purified natural lignan dimmer isolated from *Myristica fragrans* seed. World Journal of Pharmaceutical Research. 2015; 4(3):314-324.
6. Almey A, Khan AJ, Zahir S, Suleiman M, Aisyah MR, Rahim K. Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves. International Food Research Journal. 2010; 17:1077-1084.
7. Zhou YC, Zheng RL. Phenolic compounds and an analog as superoxide anion scavengers and antioxidants. Bioch. Pharm. 1991; 2:1177-1179.
8. Prasad K, Laxdal VA, Yu M, Raney BL. Evaluation of hydroxyl radical scavenging property of garlic. Mol. C. Bioch. 1996; 154:55-63.
9. Hertog MGL, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a welsh population of 95 men: the caerphilly study. Am. J Clin. Nutr. 1997; 65:1489-1494.
10. Kaur C, Kapoor HC. Antioxidant activity and phenolic content of some Asian vegetables. International Journal of Food Science and Technology. 2002; 37:153-161.
11. Olaleye MT, Akinmoladun AC, Akindahunsi AA, Antioxidant properties of *Myristica fragrans* (Houtt) and its effect on selected organs of albino rats. African Journal of Biotechnology. 2006; 5(13):1274-1278.
12. Jeong SM, Kim SY, Kim DR, Jo SC, Nam KC, Ahn DU, *et al*. Effect of heat treatment on the antioxidant activity of extracts from citrus peels. Food Chemistry. 2004; 52:3389-3393.
13. Al-Jumaily EF, Al-Shanon AA, Al-Barzanchi SI. Antioxidant and reactive oxygen species induction using purified natural lignan dimmer isolated from *Myristica fragrans* seed. World Journal of Pharmaceutical Research. 2015; 4(3):314-324.
14. Al-Reza SM, Rahman A, Young-Su Cho, Kang SC. Chemical composition and antioxidant activity of essential oil and organic extracts of *Cestrum nocturnum* L. JEOBP. 2010; 13(5):615-624.
15. Krishnaswamy T, Sellamuthu M, Subramaniam P. Antioxidant and free radical scavenging potential of leaf and stem bark extracts of *Bauhinia malabarica* roxb. Int J Pharm Pharm Sci, 2013; 5(1):306-311.

16. Politeo O, Juki M, Milo M. Chemical composition and antioxidant activity of essential oils of twelve spice plants. Croatica Chemica Acta. 2006; 79(4):545-552.
17. Gupta AD, Bansal VK, Babu V, Maithil N. Chemistry, the antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt). J Genet Eng Biotechnol. 2013; 11:25-31.
18. Shan B, Cai Y, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J Agric Food Chem. 2005; 53(20):7749-59.
19. Chakraborty P, Lavanya P, Abraham J. Bioactivity of *Myristica fragrans* methanol extract. World Journal of Pharmaceutical Research. 2015; 4(09):1155-1157.
20. Assa JR, Widjanarko SB, Kusnadi J, Berhimpon S. Antioxidant potential of flesh seed and mace of Nutmeg (*Myristica fragrans* Houtt). Int J ChemTech Res. 2014; 6:2460-8.
21. Habu JB, Ibeh BO. In vitro antioxidant capacity and free radical scavenging evaluation of active metabolite constituents of *Newbouldi anlaevis* ethanolic leaf extract. Bio. Res., 2015; 48(1):16.
22. Bor JY, Chen HY, Yen GC. Evaluation of antioxidant activity and inhibitory effect on nitric oxide production of some common vegetables. Journal of Agricultural and Food Chemistry. 2006; 54(5):1680-1686.
23. Hou JP, Wu H, Wang Y, Weng XC. Isolation of some compounds from Nutmeg and their antioxidant activities. Czech J Food Sci. 2012; 30(2):164-70.
24. Lima RK, Cardoso MG, Andrade MA, Guimaraes PL, Batista LR, Nelson DL, 2012.