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Qualitative phytochemical screening, total phenolic content and antioxidant activity in methanolic extracts of *Myristica fragrans* Houtt. (Mace)

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Mace, also known as the flower of nutmeg, belongs to the family Myristicaceae. Traditionally, mace is known to be anti-fungal, anti-depressant, aphrodisiac, digestive and carminative agent. The active components present in mace are responsible for its antioxidant properties. Qualitative phytochemical analysis showed the presence of tannins, saponins, flavonoids, terpenoids, phenolics, carbohydrates as well as proteins and amino acids. The total phenolic content of methanolic extracts of mace was 238.52 (mg Gallic acid equivalents per gram weight). The DPPH scavenging activity was 85.2% at 500µg/ml concentration, comparable to that of ascorbic acid. The present study evaluates the quantitative phytochemicals, total phenolic content and antioxidant potential of methanolic extracts of Mace, so that it can be used as complete functional food.

Key Words : Spices, Total phenolics, Phytochemicals, Antioxidant potential, Functional food

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INTRODUCTION

Spices, like vegetables, fruits and medicinal herbs, are known to possess a variety of antioxidant effects and properties (Zheng and Wang, 2001; Velioglu *et al.*, 1998; Shobana and Naidu, 2000; Madsen and Bertelsen, 1995 and Kahkonen *et al*., 1999). Spices and aromatic herbs have been used since antiquity as preservatives, colorants, and flavor enhancers (Viuda-Martos *et al.*, 2011). They may be derived from many parts of the plant: bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas and styles or the entire plant tops (Weiss, 2002). Spices exhibit a wide range of nutritional as well as medicinal benefits. Both *in vitro* and *in vivo* studies have demonstrated how these substances act as antioxidants, digestive stimulants, hypolipidemics and show antimicrobial, anti-inflammatory and anti-carcinogenic activities (Viuda-Martos *et al.*, 2011).

Mace, also known as the flower of nutmeg, belongs to the family Myristicaceae (nutmeg family). It is the deep red, lacy, net-like covering of the nutmeg seed (Raghavan, 2006). The spice is used to flavor a number of products including sauces, cakes, cookies, confectionery and candies. It provides an intense aroma to foods. Traditionally, mace has been used to treat stomach pains, dysentery, vomiting and the symptoms of malaria. It is also chewed to prevent foul breath (Raghavan, 2006). The essential oil of mace consists of a mixture of monoterpenes, monoterpene alcohols, and aromatic ethers, along with other components (Forrest and Heacock, 1972). Ground mace contains Vitamin A, phosphorus, potassium, magnesium, sodium and calcium. The essential oils and nutrients present in mace powder are responsible for its

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medicinal value.

Traditionally, mace is known to be anti-fungal, anti-depressant, aphrodisiac, digestive and carminative agent. The active components present in mace are responsible for its antioxidant properties. Argenteane, a dilignan antioxidant, has been isolated from nutmeg's mace and it possesses similar activity as Vitamin E (Calliste *et al*., 2010). The lignans present in the aqueous extract of fresh nutmeg mace showed antioxidant, radioprotective and immunomodulatory effects in mammalian cells (Checker *et al*., 2008). Kandlakunta *et al*. (2008) found that mace is a rich source of total carotenoids as well as ß-carotene. These bioactive compounds can prevent diseases and promote health by scavenging free radicals. Free radicals generated by our body adversely alter lipids, proteins and DNA and cause a number of diseases including atherosclerosis, inflammatory conditions, cancers as well as ageing (Lobo *et al*., 2010).

Since the synthetic antioxidants have been proved to be fatal for human body, there is an increasing interest in the natural products like spices and herbs with antioxidant potential. Mace (also known as Javitri in India) is obtained from the nutmeg fruit and has a sweet and spicy taste. It helps to lower blood pressure, soothe a stomach ache, and help detoxify the body (Season with spice, 2013). Scientists focused more on the nutmeg part but very less research has been done on mace. The present study evaluates the quantitative phytochemicals, total phenolic content and antioxidant potential of mace extracts so that it can be used as complete functional foods.

METHODOLOGY

Chemicals :

Ethanol, Methanol, Sodium carbonate, Gallic acid, Folin-Ciocalteu reagent and DPPH (1,1-diphenyl-2-picrylhydrazyl) were used. All the chemicals and reagents used were of analytical grade.

Collection of plant material and sample preparation :

Fresh mace was purchased from a local grocery shop in Noida, Uttar Pradesh, India. The samples were cleaned, shade dried and powdered.

Preparation of extracts :

Powdered mace was extracted with methanol at room temperature prior to removal of solvent. 10 grams of the ground sample was mixed with six times of 99.6 per cent methanol and kept for 24 hour. This process was repeated thrice and filtrates were collected. The filtrates obtained were concentrated under vacuum on a rotary evaporator (Buchi rotary evaporator, Model R-124) and stored at 4^0C for further use (Song *et al*., 2010).

Quantitative phytochemical screening :

Methanolic extracts of powdered mace were used for qualitative screening of phytochemicals as per standard biochemical procedures. The tests were performed to confirm the presence of alkaloids, carbohydrates, proteins and amino acids, glycosides, flavonoids, tannins, phenolics, terpenoids and steroids (Tiwari *et al*., 2011).

Estimation of total phenolic content :

The total phenolic content in methanolic extracts of powdered sample was estimated by Folin-Ciocalteu reagent, as described by Singleton and Rossi (1965). 100 mg of gallic acid was dissolved in 100 ml ethanol to prepare Gallic acid stock solution (1000 µg/ml). Various dilutions of standard gallic acid were prepared from this stock solution. 1 ml aliquots of 1.0, 2.5, 5.0, 10, 25, 50 and 100 µg/ml of gallic acid solution was mixed with 5.0 ml of Folin-Ciocalteu reagent (diluted ten-fold) and 4.0 ml of sodium carbonate solution (75 g/l) and calibration curve was plotted. The absorbance was measured after 30 min at 20°C at 765 nm. 1 ml extract was mixed separately with the same reagents and absorbance was measured at 765 nm after 1 hour. The total phenolic compound in all the extracts was determined using the formula :

$$\mathbf{C = C_1 \times \frac{V}{m}}$$

where, C= Total content of phenolic compounds in mg/g in GAE (Gallic acid equivalent); C_1= The concentration of gallic acid established from the standard curve in mg/ml; V=The volume of extract in ml, M=Weight of plant extract in grams.

Determination of free-radical scavenging activity by DPPH method :

The free radical scavenging activity of test samples was measured by 1, 1- diphenyl-2-picrylhydrazyl (DPPH) (Kaur and Arora, 2011). 1.5 ml of 0.1 mM solution of DPPH in methanol was added to 0.5 ml of extract solution in methanol at different concentrations (100-500µg/ml). The mixture was shaken vigorously and allowed to stand in dark for 30 min at room temperature. The absorbance was then measured at 517 nm using a spectrophotometer. A blank without DPPH was used to remove the influence of the colour of samples. A methanolic solution of DPPH was used as negative control. The DPPH radical scavenging activity was calculated using the following equation :

$$\mathbf{DPPH\ Scavenging\ Effect\ (\%) = \frac{A_0 - A_s}{A_0} \times 100}$$

A_o is the absorbance of negative control and A_S is the absorbance of sample.

OBSERVATIONS AND ASSESSMENT

Qualitative phytochemical analysis of mace extracts showed the presence of tannins, saponins, flavonoids, terpenoids, phenolics, carbohydrates as well as proteins and amino acids (Table 1).

Table 1 : Qualitative phytochemical screening of mace extracts

Sr. no.	Phytochemical	Mace extract
1.	Alkaloids	ND
2.	Proteins and amino acids	+
3.	Carbohydrates	+
4.	Phenols	+
5.	Terpenoids	+
6.	Sterols	-
7.	Saponins	+
8.	Glycosides	ND
9.	Flavonoids	+
10.	Tannins	+

+ Present, - Absent, ND - Not detected

Total phenolic content was determined using Folin-Ciocalteu reagent. Gallic acid was used as the standard compound. The standard curve of Gallic acid concentrations and absorbance is shown in Fig 1. Standard curve equation was found out to be :

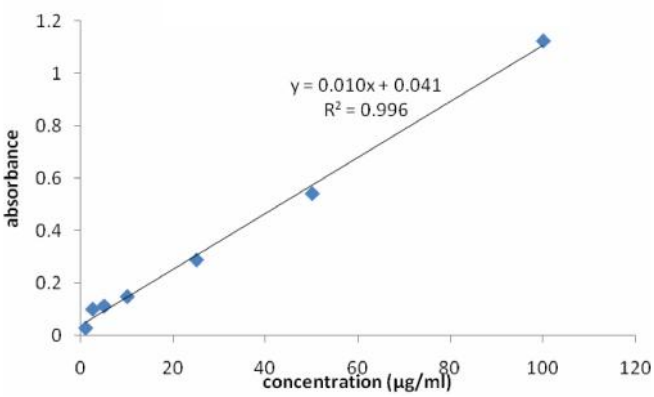

Fig. 1: Standard gallic acid curve

y = 0.0106x+0.041

R^2=0.996

The total phenolic content of methanol extracts of mace 238.52 (mg Gallic acid equivalents per gram weight) (Table 2). Data expressed as mean ± standard error of three samples analyzed separately.

Table 2 : Total phenolic content of methanol extracts of mace

Sr. No.	Sample	Absorbance at 765nm (Mean ± Standard error)	Total phenolic content (mg gallic acid equivalents per gram weight)
1.	Mace	1.34 ± 0.021	238.52

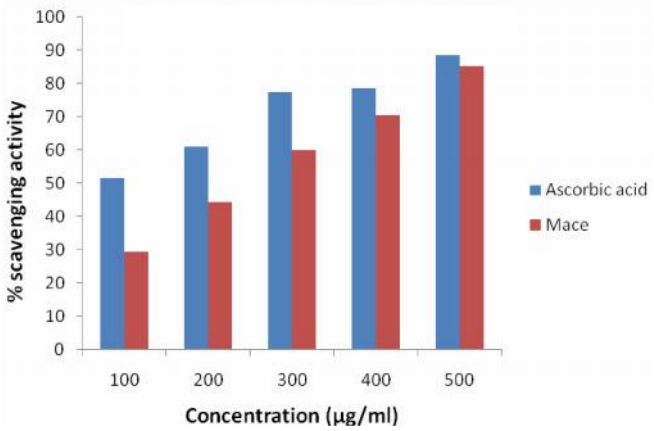

Fig. 2: Comparative analysis of per cent scavenging activity of ascorbic acid and mace extracts

The reduction capability of DPPH was measured by the decrease in its absorbance at 517 nm, due to the antioxidants. DPPH reacts with the antioxidants, accepts a hydrogen atom and gets converted to 1, 1-diphenyl-2-picrylhydrazine, thereby, showing a decrease in absorbance. The methanolic extracts of mace showed good concentration-dependent DPPH radical scavenging activity. Results are presented in Table 3.

Spices have been known to possess medicinal properties and their use in traditional systems of medicine has been on record for a long time (Srinivasan, 2005). Gupta *et al*. (2013) established the antioxidant and antimicrobial potential of nutmeg, but the complete potential of mace is yet to be explored. The total phenolic content in mace extracts was found out to be 238.52 mg gallic acid per gram weight. The DPPH scavenging activity was 85.2 per cent at 500µg/ml

Table 3 : Per cent scavenging activity in mace extracts at different concentrations

Concentration (µg/ml)	Absorbance at 517 nm		Per cent scavenging activity	
	Ascorbic acid	Mace extract	Ascorbic acid	Mace extract
100	0.252	0.582	51.5	29.11
200	0.202	0.458	61.1	44.2
300	0.118	0.329	77.3	59.9
400	0.111	0.241	78.6	70.6
500	0.06	0.121	88.46	85.2

concentration, comparable to that of ascorbic acid.

Conclusion :

Due to the lifestyle changes, chronic diseases including cardiovascular and inflammatory diseases as well as some forms of cancer are prevalent these days. Since the synthetic antioxidants are unsafe for human consumption, the interest is shifting towards natural products having antioxidant properties. Mace is one such spice, commonly used for flavoring. It contains many bioactive molecules including phenolic acids and flavonoids that can prevent diseases and promote health. Thus, inclusion of mace in the diet would be beneficial for the health due to its high antioxidant capacity. The study hence, concludes that mace has good phenolic content and antioxidant activity and therefore a complete functional food.

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