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Systematic Analysis of Essential Oil of the Two Ecological Varieties of Myristica Fragrans (Nutmeg): Interplay Between Mace and Seed

Research Article

Poresh Chandra Barman, Gulshan Ara and Shamsun Naher*
Department of Chemistry, Jagannath University, Dhaka-1100, Bangladesh

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ABSTRACT

Nutmeg (*Myristica Fragrans*) is recognized as a spice and commonly used in culinary. It is also significantly applied as medicine for its pharmacological properties such as antifungal, antimicrobial, and antioxidant. The main goals of this work are to methodical study of the foremost components of the essential oil extracted from nutmeg mace and present a comparative view for essential oil between nutmeg seed and nutmeg mace of the two ecological varieties. Essential oil has been extracted from nutmeg using steam distillation. Eleven natural oil components were identified from the essential oil of nutmeg mace for the two varieties with the help of GC-MS measurements. Among the eleven components of the essential oil seven components are common in nutmeg mace of both the varieties. The concentration of each of the common constituents of the essential oil found higher in Indian nutmeg mace than that of Sri Lankan nutmeg mace. The comparative study reveals the presence of higher concentration of natural essential oil constituents in nutmeg seed than nutmeg mace from Indian variety.

Keywords*: Myristica fragrans, nutmeg mace, nutmeg seed, steam distillation, essential oil, comparative study*

1. Introduction

Nutmeg (*Myristica fragrans*) is a bushy tropical evergreen tree about 10-20 m height, native to Indonesia, India, West Indies, and Sri Lanka and in tropical countries like South Africa (Pal *et al*., 2011, Gils *et al.*, 1994). Nutmeg is well known in ethnomedicine as carminative, nervous stimulant, aphrodisiac, and tonic (Krishnamoorthy *et al*., 2000, Chirathaworn *et al*., 2005). It is also useful for treatment of paralysis and in increasing blood circulation (Latha *et al.*, 2005, Panayotopoulos *et al*., 1970). Nutmeg holds 30-40% fats and about 13% volatile oil (Leela *et al*., 2008). Moreover, for further phytochemical examination and pharmacological study, essential oils have been isolated from *M. Fragrans*, and have been verified to show biological properties including antimicrobial, antifungal, hepatoprotective, and anti-inflammatory effects (Abourashed *et al*., 2016).

Other than as medicine, Nutmeg is commonly used in beverages (Nutmeg fruits) and as spices in sweet and salty food. Generally, there are two parts of Nutmeg, the aril (mace) and the nut (seed) (Pirase*t al*., 2012; Zhao *et al*., 2019). In addition, the essential oil was established as a good natural preservative for processed food and as a source of antioxidant (Šojić *et al.*, 2015). It is mixed with curry powder, teas and soft drinks or in milk and alcohol for its flavour and taste (Olaleye *et al*., 2006).

For many years, essential oils extracted from plants are being applied as medicine for infection in respiratory system like common cold, bronchitis, asthma (Federspiel *et al*., 1997, Boyd *et al*., 1970,

*Corresponding author: Shamsun Naher
Email: shamsunnaher2002@yahoo.com

Burrow *et al.*, 1983). Inhalation of the vapour of essential oil is also helpful to treat sinuses (Maruzzella *et al*., 1959 and 1960, Kienholz *et al*., 1959). As essential oil is volatile at room temperature, it can be applied easily as medicine through inhalation (Inouye *et al.*, 1983, Goi *et al.*, 1985).

There are sufficient sources of essential oil existing in biosphere and one of which is nutmeg. This work has been carried out to take a challenge for maximum pulling out of essential oil from the nutmeg mace. The key goal of this work is to investigate the comparative inspection of essential oil from nutmeg mace and nutmeg seed concerning of their varieties in composition and in quantity. Frequent studies on extraction of essential oil have been done for nutmeg of different geological varieties earlier other than India (Kerala) and Sri Lanka. The reports on the comparison of nutmeg seed and nutmeg mace concerning of the constituents in essential oil are very few.

In this work, essential oil has been extracted from the nutmeg mace (*M. fragrans)* of two different sources, India and Sri Lanka, and identified the chemical composition of the natural oil constituents in the extracted essential oil with the help of GC-MS measurements. Finally, a comparative study has been explored between nutmeg mace and nutmeg seed of both geographical varieties.

2. Materials and Methods

The samples (Nutmeg) origin of India (Kerala) and Sri Lanka were collected from local city market (Chawkbazar) in Dhaka. After washing and cleaning, the raw sample was kept in air to dry for 14 days. The dried samples were crushed for 1 hour by a grinder (Fritsch mortar, Germany). The crushed nutmeg was then strained to remove the abrasive parts and collected fine parts (0.25-0.50 mm) for the extraction process.

Extraction of essential oil

The essential oil was taken out from nutmeg mace by steam distillation. With 600 mL distilled water, about 200 g of nutmeg mace powder was taken into distillation flask (Clevenger's, JAOAC) for distillation. The distillate (steam and essential oil) was collected for four hours in a round bottomed flask after condensation of the vapour mixture. The distillate was suspended in ether and separated using separating funnel. The oil layer was then dried using anhydrous Na_2SO_4 and collected after filtration for removing extra ether in vacuum drier. The obtained essential oil was stored in an airtight vial. The yield of the extracted essential oil was calculated using the well-known formula reported by Rao *et al*. (2005).

Preparation of samples and GC-MS analysis

Chloroform was added to dilute essential oil (7%) before analyzing by gas chromatography-mass spectrometer (GC-MS) applying electron impact (EI) ionization technique. The flow rate of the carrier gas (helium at 90 KPa) was tuned to get repeatable retention time and lessen noise of the detector. The diluted essential oil was inserted using a micro syringe into a heated injection part(220ºC) and vaporized before passing through a column (fused silica capillary column) packing with diethylene glycol succinate (10%). The essential oil was allowed to separate into its individual components and passed to detector.

Identification of the components

National Institute of Standard and Technology (NIST) has more than 6200 patterns for analyzing GC-MS spectra. The spectrum of unidentified component was detected by relating with the spectrum of previously identified patterns of components obtained from NIST library. The molecular formula, molecular weight, and composition percentage of the components of essential oil were recorded.

3. Results and Discussion

Nutmeg mace has a distinguished pleasing odor and somewhat spicy taste. Physical properties of the essential oil obtained from both the varieties (Indian and Sri Lankan) are similar in taste, colour, and odor (Table 1). The typical strong odor and peppery taste of the essential oil pulled out from the nutmeg mace is probably because of the presence of some volatile compounds like phenol, alcohols, and esters at room temperature (Al-Shahhat, 2000 and Venturella, 2000).

Table-1: Physical properties of essential oil from Sri Lankan and Indian nutmeg mace

Physical Characteristics	**Sample sources**	
	Srilankan Mace	Indian Mace
Taste	Pungent	Pungent
Odor	Spicy	Spicy
Color	Golden yellow	Golden yellow

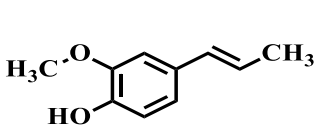
GC-MS measurements showed the chemical composition of essential oil extracted from nutmeg mace of two varieties, Indian and Sri Lankan including molecular formula and weight (Table2).

Table-2: Constituents of the essential oil from mace of *Myristica fragrans* of India and Sri Lanka.

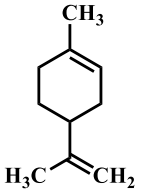
Sl. No.	% Area		Name of the compound
	India	Sri Lanka	
1.	0.09	0.08	Isoeugenol or phenol 2-Methoxy-4-(prop-1-en-1-yl)(*Z*)
2.	0.08	0.09	Limonene or cyclohexene 1-Methyl-4-(1-methylethenyl)
3.	0.28	0.27	Eugenol or phenol 2-Methoxy-4-(prop-2-en-1-yl)(*E*)
4.	0.07	0.09	Terpinolene or cyclohexene 1-Methyl-4-(propan-2-ylidene)
5.	0.03	0.03	Caryophyllene or (1*R*,4*E*,9*S*)-4,11,11-Trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene
6.	0.24	0.23	Bornyl acetate
7.	0.02	0.02	Piperitol or 2-Cyclohexen-1-ol,3-methyl-6-(1-methylethyl)
8.	0.05	0.06	Pinene or 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
9.	0.02	0.02	Carene or 3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene
10.	1.75	1.70	Methyl eugenol or benzene 1,2-Dimethoxy-4-(prop-2-en-1-yl)
11.	0.15	0.14	Copaene or α: (1*R*,2*S*,6*S*,7*S*,8*S*)-8-isopropyl-1,3-dimethyltricyclo[4.4.0.$0^{2,7}$]dec-3-ene

Eleven constituents were found from the essential oil of nutmeg mace. The essential oil extracted from nutmeg mace of Indian variety is richer in quantity of constituents than that from Sri Lankan variety. The amount of each constituent of the essential oil from Indian nutmeg mace was higher compare to that of Sri Lankan nutmeg mace.

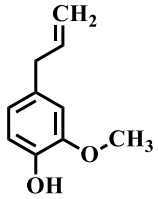
The structures of the identified constituents are as below-



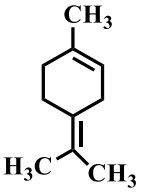
Isoeugenol



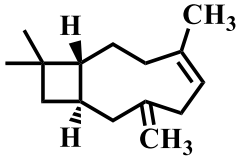
Limonene



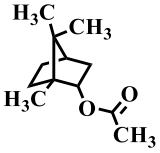
Eugenol



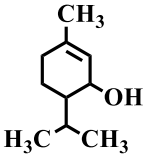
Terpinolene



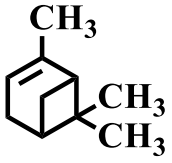
Caryophyllene



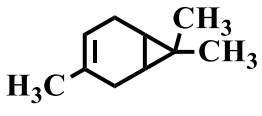
Bornyl acetate



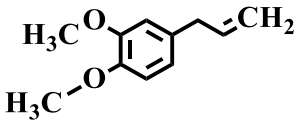
Piperitol



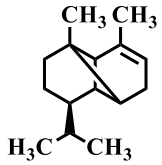
Pinene



Carene



Methyl eugenol



Copaene

Comparison between essential oils from nutmeg mace and nutmeg seed

The constituents of essential oil taken out from nutmeg seed were reported earlier (Naher *et al*., 2013). It was found that for Indian variety the essential oil shows the presence of nine constituents and for the Sri Lankan variety it shows ten constituents where seven constituents are common in both. The essential oil from Indian nutmeg seed holds 2-methyl-5-(1methyethyl)-bicyclo[3,1,0]hex-2-ene and 2,4-dimethyl-1,3-dioxolane-2-methanol which are absent in the essential oil from Sri Lankan nutmeg seed. On the other hand, 4-hydroxy-4-methyl-2-pentanone, (+)-4-carene, and 5-(2-propenyl)-1,3-benzodioxole were found only in essential oil from Sri Lankan nutmeg seed. Indian nutmeg seed showed higher concentrations of each of the seven common constituents of essential oil than that from Sri Lankan nutmeg seed apart from β-phellandrene and 1,2,3-trimethoxy-5-(2-propenyl)-benzene.

In contrast with the compositions of essential oil from nutmeg mace, essential oil from nutmeg seed showed a great distinction. Only two constituents are common in the essential oil from nutmeg seed and nutmeg mace and they are carene and pinene (Naher *et al*., 2013). In addition, the essential oil pulled out from nutmeg seed showed the presence of higher concentrated natural oil constituents than that from nutmeg mace. Furthermore, the amounts of natural oil constituents of essential oil from Indian nutmeg (both seed and mace) are better-off than Sri Lankan nutmeg.

The systematic investigation for the extraction of essential oil and identification of the natural oil constituents in the essential oil from nutmeg (both seed and mace) for the two varieties, showed the existence of a composite combination of plentiful natural oil constituents in essential oil. Most of the constituents are found in significant amount and few are in minute quantity. These great differences in the composition of essential oil from nutmeg (seed and mace) of the two varieties, are because of the variation of soil composition, weather changes, agricultural individualities, stage of maturity, biological circumstances, and geographical separation.

4. Conclusion

The essential oil obtained from the nutmeg mace for both the varieties, Indian and Sri Lankan, contains various constituents of natural oil nearly same amounts of each of the common constituents. Nutmeg seed and nutmeg mace showed a large variation in the compositions of natural oil constituents in the essential oil and also in the concentration of the constituents. Indian nutmeg seed comprises higher concentration of natural oil constituents of essential oil than nutmeg mace of the two varieties including Sri Lankan nutmeg seed. The higher concentration of the natural oil constituents of essential oil was found in Indian nutmeg seed and mace compared to both parts of nutmeg of the Sri Lankan variety, respectively. The presence of natural oil constituents make nutmeg potentially beneficial for both foods and medicine**.**

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