Extraction and Purification of Terpenes from Nutmeg (*myristica fragrans)*

Essam F. Al-Jumaily and Maytham H. A. Al-Amiry
Department of Biotechnology, Genetic Enginerring and Biotechnology Institute for Post Graduate, Baghdad University, Baghdad-Iraq.

Abstract

This study was conducted with the aim to extract and purify the essential oil containing terpenes from the dried seeds of nutmeg *myristica fragrans* available in Iraqi markets.The essential oil was extracted by steam distillation method with a yield of 4.7 – 7.5 gm / 100 gm, and the resultant oil was subjected to sensory and physical evaluation. It has a special spicy odor, slightly pungent taste, colorless to pale yellow in color, not dissolved in water but dissolved in organic solvents like ethanol, hexane, chloroform and ether. The specific gravity, refractive index and optical rotation were (0.890 gm / ml), (1.4822) and (+22˚), respectively. Gas chromatography is used for the quality and quantity evaluation of the essential oil constituents which revealed the presence of 49 volatile compounds. The methanolic extract obtained by reflux the seeds powder with 70% methanol in soxhlet apparatus and yield 12.8%. The extract was subjected to phytochemical detection which confirms the presense of alkaloids, terpenes, flavonoids, glycosides, tannins and resins, with the exception of saponins and coumarines which gave negative tests. Myristicin was purified from the methanolic extract of nutmeg dried seeds, which were detected in the essential oil by TLC and vanillin-H2SO4 reagent. The purified myristicin was obtained after the application of adsorption chromatography on silica gel column and detected on T.L.C. plate with standard myristicin.

Both the essential oil and purified myristicin were subjected to gas chromatography which showed the presence of myristicin in the oil in a concentration of 6%.

Keywords: Nutmeg, *myristica fragrans*, myristicin, methanolic extract.

Introduction

Nutmeg (*Myristica fragrans* Houtt.) belongs to the family Myristicaceae, with about 18 genera and 300 species. The genus *Myristica* is distributed from India and South-east Asia to North Australia and the Pacific Islands. Three species occur in India, including *M. fragrans*, *M. beddomeii* and *M. malabarica* [1].

Nutmeg (NM) has its origins in the Spice Islands of Indonesia, it is indigenous to the Banda islands (Maluku or Moluccas islands) in Indonesia, formerly known as the spice islands, it is also cultivated in the Caribbean, south India, Sri Lanka, Sumatra, and Malaysia, It has been widely popular in Europe and India for its flavoring and medicinal properties [2,3].

The traditional use of nutmeg include the treatment of rheumatism, cholera, psychosis, stomach cramps, nausea, diarrhea, flatulence and anxiety in addition to use as aphrodisiac and abortifacient [2].

In traditional medicine, nutmeg and nutmeg oil have been used for illnesses related to the nervous and digestive systems, It is used in both Western and Chinese herbal medicine, It has been said to relax the muscles, remove gas from the digestive system, sedate the body, and to be of value for such stomach problems as indigestion, It is also used for chronic nervous disorders, to prevent nausea and vomiting, and for kidney disorders, and in Chinese medicine is used for diarrhea, inflammation, abdominal pain, and liver disease, among other aliments [4].

In Indochina, powdered seeds in boiled rice are used as a remedy against dysentery, anorexia, and colic. It is further used to treat malarial debility. In Indonesia, mace is also used as an analgesic and as medicine for rheumatism [5].

The essential oil content in nutmeg from South India ranges from 3.9 to 16.5%, whereas in mace it varies from 6 to 26.1% [6].

It must be noted that the composition of distilled volatile oil is not identical to the natural oil in the kernel or oleoresin extract. The kernel consists of 30–55% oil and 45–60% solid matter, the volatile oil accounts

for 5–15% of the nutmeg kernel, while the fixed oil accounts for 24–40% [7].

Essential oil yield and composition of nutmeg and mace collected from Grenada revealed that the quality of the two oils was very similar, but they showed variation in the quantity of the components. The nutmeg oils showed 85–93% monoterpene hydrocarbons, 6.6–12% oxygenated monoterpenes and sesquiterpenes and 3.5% aromatic ethers, while the corresponding values for the mace oils were 75–94%, 4.7–17.6% and 0–5.9%, respectively [7]. Myristicin is a naturally occurring benzodioxole compound (phenylpropan derivative) found in anise, Star anise, black pepper, carrot, common fennel, mace, nutmeg, sweet fennel, many natural oils, and flavoring agents [8]. Myristicin, or methoxysafrole, is the principal aromatic constituent of the volatile oil of nutmeg, it is a natural oraganic compound present in small amount in the essential oil of nutmeg [9]. Myristicin is present in plant from carrot family (Umbelliferae) including dill, celery, parsnip, parsley and carrot, this compound is also a minor constituent of oil of black pepper (*Piper nigrum*) [2]. The present study aims to extraction and purification of the essential oil containing terpenes from nutmeg seeds.

Materials and Methods

Collection of samples

The dried seeds of myristica fragrance were collected from local market in Baghdad during September / 2009 and identified by the botanist professor Dr Ali Almosawi In the college of sciences / Baghdad University.

Isolation of essential oils by steam distillation method

The plant material extraction was carried out according to Koedam[10] by using a steam generation source, the water and essential oil. After that the water essential oil mixture was transferred to a separatory funnel and kept in cooled place over night, the oil layer which was in the top could be then collected, dried over anhydrous sodium.

Isolation of essential oils from nutmeg using Clevenger

The hydro distillation method had been used for the extraction of essential oils from nutmeg. Nutmeg which is the kernel of *myristica fragrans* were grounded into fine powder. The distillation flask of 500 ml contained water about 2/3 of its volume and 50 gm of the powder.The operation proceeded by heating the flask at 100˚ C, heat was applied to the flask and the volatile oil was carried with the steam to a cold condenser, the lighter oil rises to the top of the separator. The essential oils collected was dried over anhydrous sodium sulphate, weighed and stored in a sealed vial dark colored at 4˚ C. The yield percentage of essential oil was determined using the formula described by Rao *et al.*[11].

$$\text{Yield (\%)} = \frac{\text{Amount of essential oil recovered (g)}}{\text{Amount of plant material distilled (100g)}} \times 100$$

Evalution of some physical characteristics of nutmeg seeds essential oils:

The physical properties odor, colour, taste, specific activity, refractive index and optical rotation were evaluated according to the methods mentioned by Guenther [12] and Al-Shahhat [13].

Preparation of the nutmeg methanolic extract:

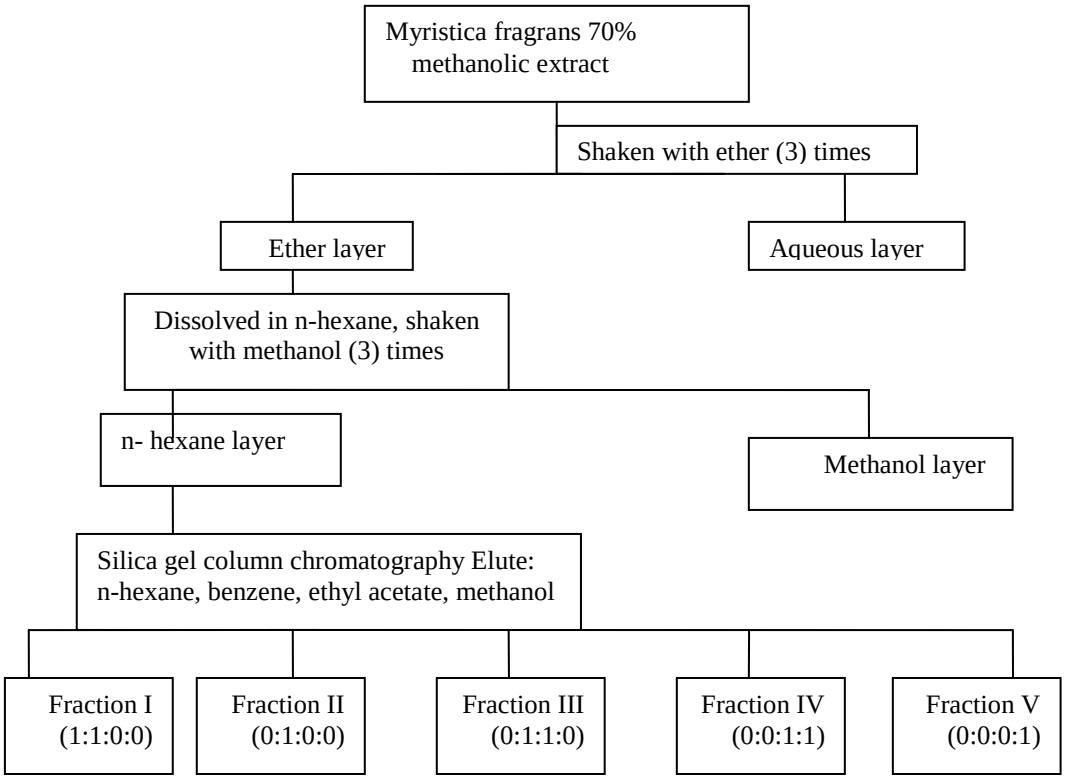
According to Ozaki *et al*. [5] with some modification. 50 gram of the crude powder of seeds was refluxed with 350 ml of 70% methanol (1:7) in soxhlet apparatus for 8 hours. The solution was filtered through a filter paper and evaporated to dryness under vacuum at 40˚C, the dried extract was weighed.

Liquid –liquid partition

The dried methanolic extract was dissolved in ether and transferred to a separatory funnel extracted with water three times in a separatory funnel; then D.W was added in a quantity equal to the ether phase, gently shaken and the process was repeated three times, the ether phase was separated and evaporated to dryness under vacuum at 40˚C. The ether soluble fraction was dissolved in n-hexane and extracted with methanol three times in the same manner. The n-hexane phase was separated and evaporated to dryness under vacuum at 40˚C. The n –hexane soluble fraction was chromatographed on a silica gel column,

Column Chromatography (Partial Purification) by Solid – Liquid Adsorption Chromatography

A partial purification of myristicin from the n-hexane phase preceded using open glass column (2.5 x 21) cm filled with silica gel G200 special for column chromatography. The residue was dissolved in 1-2 ml hexane and the mobile phase was n-hexane: benzene: ethyl acetate: menthol, 1:1:1:1. as shown in (Scheme (1)). [5].



Scheme (1) Flow diagram of fractions of the nutmeg methanol extract on the silica gel column.

The elusions were collected for each of mobile phase used and numbered as fractions; all fractions were tested on T.L.C. plates for the presence of myristicin. Only the positive results elution were collected and dried under vacuum by a rotary evaporator. The myristicin spots were detected on a TLC aluminum sheet silica gel $60F_{254}$ in comparison with the standard spot using the same mobile phase in the column chromatography.

The R_F values (mobility relative to solvent front) were measured to represent the distance; a compound moved in chromatography relative to solvent front [14]. The collected elution after dryness was referred to as "partial purification" for myristicin.

Preparative thin layer chromatography

This method was described by Badheka *et al*. [15]. It involved using glass plated –58-attention: (20x20 cm) coated with silica gel with thickness of 1 mm type (60F254). The separation solution (mobile phase) consisted of (ethyl acetate: toluene) (7: 93). when the solvent system moved about 15 cm from the spots, then pulled out and left to dry in air.

The spots were scratched and plate was washed by diethyl ether, centrifuge at 3000 rpm for 20 minutes at 45˚C, the supernatant was filtered and then concentrated by rotary evaporator. The examination of the thin layer chromatography which was repeated by taking a small amount of the concentrated solution of diethyl ether, proved the purity of myristicin as showed in an overview.

The R*f* value was calculated according to this equation:

R*f* = distance of sample / distance of solvent.

General tests for phytochemicals

General tests for phytochemical compounds in the essential oils and myristicin were determined by the following tests; detection of terpenes and steroids according to

Al-Maisary,[16]; detection of tannins and resins according to Shihata,[17]; detection of phenols according to Harbone, [14]; detection of saponins and alkaloids according to Harborne,[18]; detection of glycosides according to Evans, [19]; detection of coumarines according to Geisman, [20] and detection of Flavenoids according to Jaffer *et al*.[21].

Analysis of the oil

Gas Chromatography analysis was carried out using Shimadzu GC-14A gas chromatograph with FID detector and SE-30 column (length and inner diameter). The operating conditions was as the follow : carrier gas was He + H2 + air (20 ml/min constant flow), the oven temperature for first 2 min was 100˚C and then increased at a rate of 10˚C/min until 270˚C hold for 2 min, injector and detector temperature were set at 250˚C and 300˚C respectively.

Results and Discussion

The essential oils collected from all the plant seeds of *myristica fragrans* was less dense than water and exhibited a colorless or pale yellow color.

The relative amount (g / 100g powdered material) of essential oil extracted from the plant dried seeds has been ranged from 4.70 - 7.50 gm/100 gm of powdered seeds depending on the quality of the seeds, however, the latter quality is the most suitable for distillation due to low content of fatty acids [22]. This amount of oil is approximately in agreement with Leela [7] also Jukic *et al* [23] have reported the yield of oil as 4.92 g / 100 gram and Spricigo *et al*.[24] have mentioned the yield of oil as 6.9 gram / 100 gram while Rahman *et al*.[25] have showed that the yield has been 2.28 g/100 gm. These differences in the amount of oil extracted are due the variation in origin; soil; climate and quality of the seeds used and whether they are wormy or sound [24; 7].

Sensory Evaluation for the essential oil

Table (1) shown the sensory evaluation carried out on the essential oils from the dried seeds of *Myristica fragrance*.The odor of the essential oil obtained is strong, turpentine like odor or spicy. Each essential oils is characterized by a special odor attributed to the presence of some low molecular weight compounds like alcohols, esters, phenols and oxygenated compounds which have the future of being highly volatile at room temperature [13].

The odor of the essential oils is regarded as one of the important diagnostic futures, because every essential oil has its own special odor and hence regarded as a diagnostic tool for the plant that contain the oil [26].

Table (1)
The sensory evaluation of nutmeg seeds essential oil.

Characteristic	*Observation*
Color	Colorless or pale yellow
Clarity	clear
Odor	Turpentine-like or spicy
Odor intensity	Strong
Solubility	Insoluble in water, soluble in ethanol, petroleum ether, diethyl ether, chloroform

The essential oil of nutmeg seeds are characterized by a colorless to pale yellow color. Gopalakrishnan [27] has reported that the color of the East Indian nutmeg oil has been colorless to yellow while that of West Indian has been pale yellow.

The essential oil of nutmeg seeds is characterized by a strong spicy taste. All the essential oils are characterized by a taste ranging from sweaty, astringence and burning. [26].

Guenther [22], has reported that the specific gravity of East Indian nutmeg oil is ranging from 0.880 to 0.913 at 25˚C, while the specific gravity for west Indian nutmeg oil is 0.859 to 0.865 at 25˚C. Sarath-kumara *et al*.[28] have mentioned that the specific gravity of the East Indian Nutmeg oil ranges from 0.885 to 0.915 and that of west Indian has been 0.860 to 0.88 at 20˚C, Leela [7] has also report that the specific gravity of east Indian oil is ranging from 0.880 to 0.913 and that of west Indian is ranging from 0.859 to 0.865. Reineccius [29] have reported that the specific gravity of east Indian has been 0.880 – 0.910 and of west Indian has been 0.854-0.880 at 25˚C.

Generally, the specific gravity of all essential oils extracted from plant material is ranging from 0.824 to 1.172 [26; 13], this is attributed to the genetic factors.

The refractive index of the nutmeg essential oil is found to be 1.4822, Leela [7], has reported that the refractive index of the East Indian Nutmeg essential oil is ranging from 1.4776–1.4861,while that of West Indian Nutmeg essential oil is ranging from 1.4729 - 1.4746. Also the value is in the range of refractive index of East Indian nutmeg oil mentioned by Sarath-kumara *et al* [30].

The essential oil of the nutmeg seeds gives a value of +22˚ For optical rotation which means it is dextrorotary oil (rotating plane polarized light clockwise), the optical rotation for nutmeg seeds essential oil if differ according to the origin and quality and it is ranging from +8.0 to + 45.0 [31].

This value of optical rotation agree with Leela [7] and Guenther [12], who report the optical rotation for East India nutmeg oil ranges from +7.53 to + 22.10 while that of west Indian ranges from +25.45 to +38.32.

Reineccius [29] had mentioned a value of +8˚ to +30˚ for the East Indian essential oil while value of +25˚ to +45˚ for the west Indians one.

Preparation of the Methanolic Extract

The total amount obtained by alcoholic extraction in this study from the use of 500 gm of *myristicas frsagrasns* seeds in soxhlet apparatus was 61.36 gm. i.e. 12.8% of dark brown oily sticky extract.

Chirathaworn *et al*. [32] mentioned that the 80% methanolic extract was 3.34%. Tajuddin *et al*. [33], mentioned that 50% ethanolic extract of nutmeg seeds was 21.20%.

The alcoholic solution (70% methanol) is used in the present study for extraction of flavenoids, terpenes, alkaloids, saponins, glycosides and tannins, after filtration, the methanolic extract has been subjected to evaporation to get alcohol free extract to avoid the harmful effect of methanol on the liver which may affect the biochemical[34].

Phytochemicals detection

The phytochemical detection of the methanolic extract shows the presence of flavenoids, alkaloids, tannins, terpenes, phenols, glycosides and the absence of saponins and coumarines as shown in (Table (2))

Table (2)
The phytochemical constituents of methanolic extract.

Active compounds	*Result*
Tannins	(+) white gelatine ppt. (+) bluish green color
Flavenoids	(+) yellow color
Alkaloids	(+) white precipitate (+)brown precipitate (+) yellow precipitate (+)orange precipitate
Saponins	(-) thick foam white precipitate(-)
Terpenes and steroids	(+) bonny color
Phenols	(+) bluish green color
Coumarines	(-) shiny green yellow color
Glycosides	(+) red precipitate
Resins	(+) turbidity

The phytochemical findings agree with Olaleya *et al*.[35], who have reported the presence of alkaloids, glycosides flavenoids, The presence of sugars, phenols, tannins agree [27]. Olaleya *et al*, [35] have reported the presence of saponins and the absence of tannins; this could be due to the difference in the extract used because they used water extract for the evaluation of active components.

Thin layer chromatography (T.L.C)

Fig.(1) shown the results that all samples of the essential oils contained Myristicin which appear as a brown zone in the upper third of the plate with R*f* 0.9 as shown in (Fig. (4.1)) [36].

Fig.(1) TLC of the essential oil in toluene: ethyl acetate phase (93:7) the brown zone indicates the presence of myristicin [36].

Preparative TLC (P.T.L.C)

Spots were scratched by spatula and monitored by TLC to know the purity of myristicin through a specific solvent system (benzene or hexane -chloroform). This test has shown that the myristicin has a high degree of purity as in (Fig.(3)).

Extraction of myristicin from nutmeg seeds

The Procedure for extraction and purification was concluded from Ozaki *et al*.[5].

According to Harborn [14], general process for plant extraction, the nutmeg seeds were extracted with 70% methanol. Alcohol, in any case, is a good all- purpose solvent for preliminary extraction.

Separation and Purification of Myristicin

Separation by Partition (partial purification) liquid/liquid

Separation of myristicin from another compound is carried out by many steps, which include (Liq/Liq) separation. This process depends on the difference in degree of polarity among compounds, which play a key role in the degree of dissolving these structures in two differed solvents in its polarity.[37].

Therefore when using (diethyl ether: water), myristicin has weak polarity. [38], myristicin will dissolve in ether while other compounds will dissolve in water, the end product after concentration by rotary evaporator at 40˚C is a pale yellow. The concentration of ether layer by rotary evaporator is a strong indicator that many related compounds have been removed. The repeated washing increases the purity of myristicin, because in first time both solutions reached were saturated with substances [5].

The resultant fraction of n-hexane and methanol were applied to TLC silica gel 60 F_{254} plate using the mobile phase benzene which gave a big spot of brown to purple in color of R*f* 0.5 and smaller spots(Fig.(2)). The big spot indicated the presence of myristicin as mentioned by Harborn [14]. The dried collection of n-hexane fraction was designated as "partial purified Myristicin".

Ozaki *et al*. [5] have been used this method for the purification of myristicin by using solvent system (benzene: chloroform: ethyl acetate) (10:10:1), spots of fraction II on the plate have been detected by Ozaki *et al* [5], under UV light and fraction II showed a big spot and another small spot, the big spot of R*f* 0.6 and identified as myristicin by the authors.

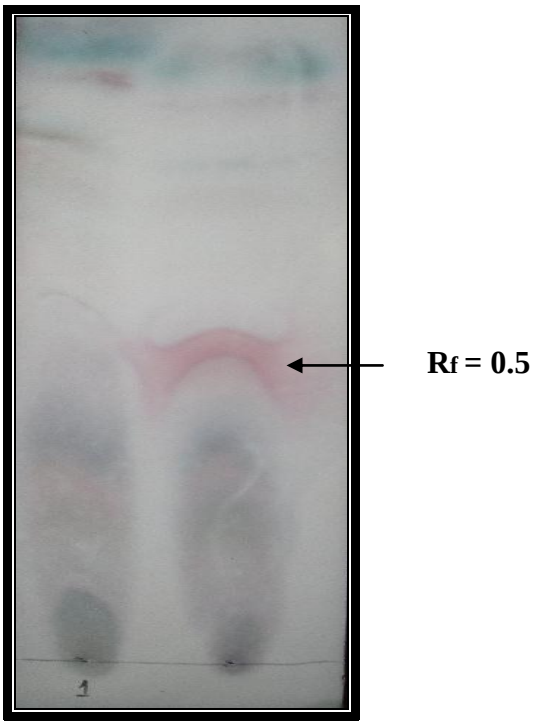


Fig.(2) T.L.C for methanolic fraction (1) and n-hexan fraction (2) (mobile phase, benzene) the big brown to purple spot of Rf 0.5 indicate the presence of myristicin [14].

Myristicin Purification

It is done using silica gel G60 column chromatography technique with a mobile phase n-hexane: benzene: ethyl acetate, methanol to elute fractions according to their affinity to mobile phase. The resultant fractions that give positive ferric chloride test 1% solution (fraction II) are detected by T.L.C silica gel 60 F_{254} plate of 0.75 mm thickness using the mobile phase n-hexane-chloroform (3:2). The TLC chromatogram in (Fig.(3)) for the fraction of the positive result (FII) and the standard have given one brown spot of R*f* 0.71 in n-hexane-chloroform (3:2) as mentioned by Harbor [14]. The dried collection was designated as "purified Myristicin". The liquid-adsorption technique was suggested by [39].

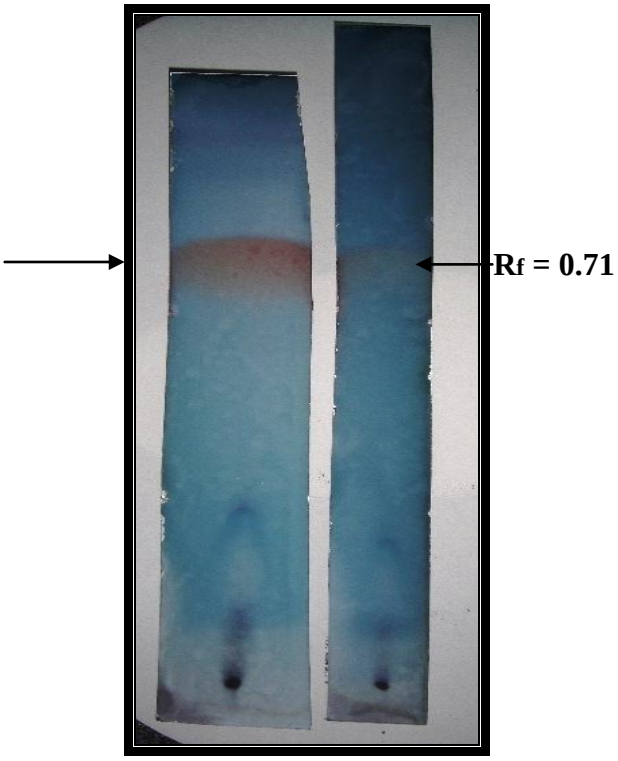


Fig. (3) T.L.C. of the standard Myristicin (left) and the purified Myristicin (right) in n-hexane - chloroform mobile phase, the brown spot of Rf 0.71 indicate the presence of myristicin [14].

Gas Chromatography (GC) for Essential oil and Myristicin

The GC analysis of the total essential oil fraction obtained during 6 hours steam distillation shows the presence of 49 peaks with their concentration and retention time (Fig.(4)). The identification of suspected peaks is shown in (Table 3). From these observations, it can be concluded that major components of the essential oil are α- pinene, β-pinene, sabinene, Myrecene, limonene, β–phelnadrene, γ-terpinene, Cymene, Terpenene–4–ol, safrole, elimicein and myristicin.

The Gas Chromatography chromatogram for the purified Myristicin as in (Fig. (5)) shows the presence of one main peak of conc. of 97.6%. (Table (4)).

According to the GC analysis of the essential oil and the purified myristicin, the percentage of myristicin in the essential oil was about 6%. Therefore it can be assumed that the nutmeg is from East India [29; 23]. Although the seeds are imported to Iraq from India but India itself imported it from south-east Asian countries because nutmeg trees are not grown to any large extent in India [40].

Table (3)
The major components of the essential oil from nutmeg.

Retention time (min.)	*Conc.*
9.705	9.6185
10. 046	21.97
10.452	8.0016
10.614	7.9689
10.896	6.1218
11.246	3. 1257
12.452	2.9515
15.127	1.1529
15.76	3.90
16.65	1.6831
16.996	1.3993
21.586	6.0177

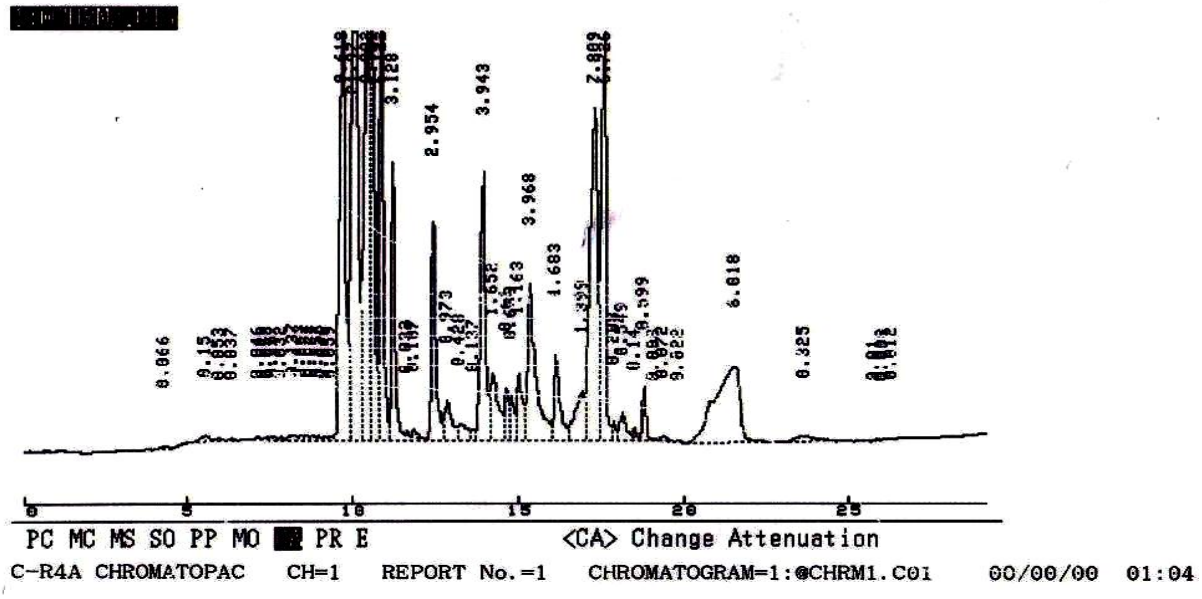


Fig.(4) The Gas chromatography analysis of the essential oil from nutmeg by using temperature programming from 100-270 ^{o}C.

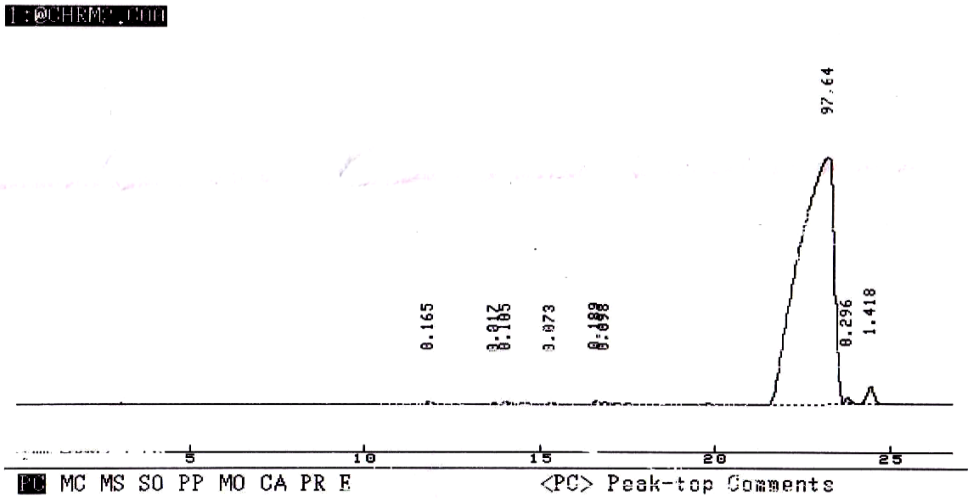


Fig.(5) The Gas chromatography of the purified Myristicin after T.L.C. by using temperature programming from 100-270 ^{o}C.

References

[1] Anon. The Wealth of India Raw Materials, Volume 6. CSIR, New Delhi, 1962, pp. 473–480.

[2] D.G.Barceloux, Medical Toxicology of Natural Substances: foods, fungi, medicinal herbs, plants, and venomous animals. *John Wiley and Sons Inc.* U.S.A., 2008. pp: 67-70.

[3] S.Raghavan, Handbook of Spices, seasonings and flavoring, 2nd edition. *CRC Press*. USA..2007. Pp: 142-143.

[4] L. DeMilto, and R.J. Frey, In: J. L. Longe (ed.), *the Gale Encyclopedia of Alternative Medicine*. Farmington Hills, Mich: Thomson/Gale. 2005.

[5] Y. Ozaki, S. Soedigdo, Y.R. Wattimena and A.G.Suganda, Antiinflammatory effect of mace, aril of *Myristica fragrans* Houtt.

and its active principles. *Japan J. Pharmacol.*, 1989.49, 1989. 155-163.

[6] K.M. Maya, T.J. Zachariah, and B.Krishnamoorthy, Chemical composition of essential oils of nutmeg (*Myristica fragrans* Houtt.) accessions. *Journal of Spices and Aromatic Crops* 13(2), 2004.135–139.

[7] N.K. Leela, Nutmeg and mace. In: Chemistry of Spice. (eds.) V.A. Parthasarathy, B. Chempakam and T.J. Zachariah., UK. *Biddles Ltd*, King's Lynn. 2008. Pp : 165-189

[8] H.G. Jeong and C.H.Yun, (1995) Induction of rat hepatic cytochrome P450 enzymes by myristicin. *Biochem. Biophys. Res. Commun*., 217(3), 2008. 966-971.

[9] B.K.Lee, J.H. Kim, J.W.Jung, J.W.Choi, E.S.Han, S.H.Lee, K.H.Ko, and J.H.Ryu, Myristicin induced neurotoxicity in human neuroblastoma SK-N-SH cells. *Toxicology Letters.* 157(1), 2005. 49–56.

[10] A. Koedam, Some aspects of essential oil preparation. In: Sandra P, Bicchi C (eds) capillary gas chromatography in essential oil analysis. Huethig, Heidelberg,1987, pp 13-27.

[11] B.R.R. Rao, P.N. Kaul, K.V. Syamasundar and S. Ramesh, "Chemical profiles of primary and secondary essential oils of palmarosa (*Cymbopogon martinii* (Roxb.) Wats. var *motia* Burk.)." *Industrial Crops and Products* 21(1): 2005. 121-127.

[12] E. Guenther, Essential oils. Vol. I., R.E. *Robert E Krieger publishing company*, New York, USA. 1972. p.18.

[13] N.A. Al-Shahat, the Volatile Oils. 1st edition. *Arabic House for Publishing and Distribution*. Egypt. 2000. (In Arabic).

[14] J.B. Harborn, Phytochemical Methods. 2ed. *Chapman and Hall*. UK. 1984. PP: 288.

[15] L.P. Badheka, B.R. Prabhu and N.B. Mulchandani, Dibenzylbutane tyrolactone lignan from Piper *Cubeba.* Phytochemistry, 25(2): 1986. 487-489.

[16] M. Al-Maisry, Effect of oil and alcoholic extract of *Azdirachta indica* on some pathogenic fungi of plant. M.Sc.Thesis. Science College, Al-Mustansria University.1999.p:120.

[17] I.M. Shihata, A pharmacological study of *Anagallis arvensis*. MSc. Thesis, Faculty of Vet. Med. Cairo Univ. Egypt. 1951.

[18] J.B. Harbone, Phytochemical Methods. A guide to modern techniques of plant analysis. *Chapman and hall*, London, New York.1973.

[19] W.C. Evans, Trease and Evans Pharmocogensy. 14thed. *W.B. Sannders Company Ltd*., London.1999.

[20] T.A. Geisman, Chemistry of flavonoid compounds. *Macmillan Co*., New York.1962.

[21] H.J. Jaffer; M.J. Mahmod; A.M. Jawad; A.Naji andA. AL-Naib, Phytochemical and biological screening of some Iraqi plants fitoterapia Lix. 1983. 299.

[22] E. Guenther, *The Essential Oils*, Volume 5. *Robert E. Krieger Publishing Company*, New York, USA. 1952. pp. 59–81.

[23] M.Jukic, O. Politeo, and M. Milos, Chemical composition and antioxidant effect of free volatile aglicones from nutmeg compared to its essential oil. *Croatica Chemica Acta* 79, 2001. 209–214.

[24] C.B. Spricigo, L.T. Pinto, A. Bolzan and A.F. Novis, Extraction of essential oil and lipids from nutmeg by liquid carbon dioxide. *Journal of supercritical fluids* 15; 1999. 253-259.

[25] A. Rahman, M.I. Choudhary, A. Farooq, A. Ahmed, M.Z. iqbal, B. Demirci, F. Demirci and K. husnu can baser, Antifungal activities and essential oil constituents of some spices from Pakistan. *third international electronic conference on synthetic organic chemistry (ECSOC-3*), 1999. September 1-30.

[26] V.S.Venturella, (Natural products In: Remington: The science and practice of pharmacy. (Edt, Gennaro, A.R.)20th ed. Lippincott, *Williams and Wilkins*. USA. 2000. PP 434-436.

[27] M. Gopalakrishan, Chemical composition of nutmeg and mace. *Journal of Spices and Aromatic Crops*, 1(1): 1992, 49–54.

[28] S.J. Sarath-Kumara, E.R. Jansz and H.M. Dharmadsa, Some physical and chemical characteristics of Sri Lankan nutmeg oil. *J. Sci. Food Agric.* 36, 1985. 93-100.

[29] G. Reineccius.Source Book of Flavors. 2nd Edition.*Aspen Publishers*.USA. 1999. pp: 305-306.
[30] Sarath-Kumara S.J., Jansz E.R. and Dharmadsa H.M. (1985). Some physical and chemical characteristics of Sri Lankan nutmeg oil. *J. Sci. Food Agric*. 36, 93-100.
[31] J.W. Purseglove, E.G. Brown, C.L. Green and S.R.J. Robbins, Spices. Vol. 1. Longman, London, 1981. pp. 174–228.
[32] C.Chirathaworn, W. Kongcharoen-suntorn, T. Dechdoungchan, A. Lowanitchapat, P. Sa-nguanmoo, Y. Poovorawan, *Myristica fragrans* Houtt. Methanolic extract induces apoptosis in a human leukemia cell line through S1RT1 mRNA downregulation. *J. MED. Assoc. Thai*. Vol.90 No. 11. 2007. P. 2422-2428.
[33] A.S. Tajuddin ; A. Latif, I.A.Qasmi and K.M. Yusuf amin, An experimental study of sexual function improving effect of *Myristica fragrans* Houtt. (Nutmeg). *BMC Complementary and Alternative Medicine.* 5: 2005. pp16.
[34] A.M. Said, The hepatoprotective activity of fenugreek seeds extract against carbon tetrachloride induced liver toxicity in rats. M.Sc. Thesis, college of pharmacy/ Baghdad University.2005. pp. 120
[35] M.T.Olaleye;C.Afolabi,;A. Akinmoladun; A. Akindahunsi, Antioxidant properties of *Myristica fragrans* Houtt. and its effect on selected organs of albino rats. *African Journal of Biotechnology*. Vol. 5 (13), 2006. pp. 1274- 1278.
[36] H.Wagner and S.Bladt, Plant Drug Analysis.2nd edition. *Springer*. Germany. 1996.
[37] Z.Y.Mohammed, Study the effect of polyphenolic compounds extracted from grape skin fruit *vitis vinifera* on some cell lines (*in vitro*). MSc. Thesis. Genetic Engineering and Biotechnology institute for postgraduate studies. University of Baghdad.2005.
[38] STN International, HODOC (CRC Handbook of Data on Organic Compounds) Database, *Columbus*, OH, 1997. Searched 5/97.
[39] R.L.P. Cannell, Natural Products isolation. *Humana Press*. New Jersey. 1998.
[40] R.Singh, Psychoactive Medicinal Plants: hallucinogenic and narcotic drugs. *Global vision publishing house*. India. 2006. pp: 206-209.

الخلاصة

هدفت الدراسة الحالية الى استخلاص وتنقية الزيت الطيار الحاوي على التربينات من البذور الجافة لنبات جوز الطيب *myristica fragrans* الموجود في الاسواق العراقية. تم استخلاص الزيت الطيار بطريقة التقطير البخاري وتراوحت نسبته من ٧.٤ الى ٥.٧ غرام/ ١٠٠ غرام بذور اعتمادا على نوعية البذور المستخدمة. وتميز هذا الزيت برائحته ولونه الاصفر الباهت وطعمه اللاذع وهو لايذوب في الماء ولكن يذوب في بعض المذيبات العضوية مثل الايثانول والهكسان والكلوروفورم والايثر. وبلغت كثافته النوعية ٠.٨٩٠ غرام لكل مل، ومعامل انكساره ١.٤٨٢٢ في حين اعطى قيمة للدوران الضوئي بلغت (+٢٢).

قدرت نوعية وكمية بعض مكونات الزيت الطيار باستخدام جهاز كروموتوغرافي الغاز فوجد انه يحتوي على ٤٩ مركب طيار. تم الحصول على المستخلص الميثانولي ٧٠% بواسطة جهاز السوكسليت وبلغت نسبته ١٢.٨%. تم اجراء الكشف الكيمائي النوعي عن بعض المركبات الفعالة في المستخلص الميثانولي،اذ بينت النتائج وجود القلويدات والسكريات والتربينات والراتنجات والفينولات والفلافونات والتانينات باستثناء الصابونينات والكومارينات التي اعطت كشفا سالبا.

كذلك هدفت الدراسة الى تنقية المايرستسين من المستخلص الميثانولي لبذور نبات جوز الطيب التي تم الكشف عن وجودها في الزيت الطيار ايضا بواسطة كروموتوغرافي الطبقة الرقيقة باستخدام الكاشف (vanillin-H_2SO_4)، وبعد اجراء عملية كروموتوغرافيا الادمصاص على عمود من الـ silica gel وباستخدام كروموتوغرافي الطبقة الرقيقة التحضيرية تم الحصول على المادة النقية وتم التعرف عليها عن طريق كروموتوغرافي الطبقة الرقيقة مع المادة القياسية.

تم التعرف على نسبة المايرستسين في الزيت الطيار عن طريق كروموتوغرافي الغاز لكل من الزيت الطيار والمايرستسين المنقى حيث تطابقت حزمة الاخير مع الحزمة التي اعطت تركيز ٦% في الزيت الطيار.