ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Issue 3, 2014

ISSN - 0974-2441

Original Article

GC MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *MYRISTICA FRAGRANS* SEED EXTRACTS AGAINST LOWER RESPIRATORY TRACT PATHOGEN *ACINETOBACTER BAUMANII*

[1]T.P.KUMARI PUSHPA RANI, S.K.SUNDAR[*] AND [1]B. VIJAYALAKSHMI AMMA

[1]Centre for Biological Sciences, Noorul Islam University, Kumaracoil, [*]Department of Microbiology, M.R.Government Arts College, Mannargudi. Email: sksundar@yahoo.com

Received: 5 June 2014, Revised and Accepted: 27 June 2014

ABSTRACT

This study is destined to evaluate the antimicrobial activity of Nutmeg (*Myristica fragrans* Houtt) seed extract against the lower respiratory pathogen (*Acinetobacter baumannii*). The extract was subjected to phytochemical detection which confirms the presence of alkaloids, terpenoids, flavonoids, phenols and quinones, with the exception of Phlobatanins. The seed extract was tested against sixteen isolates of *Acinetobacter baumannii.* The susceptibility of these isolates towards the seed extracts was compared with each other and the antibiotic (Meropenem) which was used a as a positive control. Results in the former showed that toulene, tetrahydrofuran and methanol extracts of the seed exhibited antibacterial activity against the bacterial isolates The bioactive components present in the methanol extract were also evaluated by GC-MS analysis and the analysis revealed the presence of thirteen compounds.The study concludes that the secondary metabolites present in the plant can be an indispensible source of anitimicrobial compounds.

Keywords: *Myristica frangrans*, *Acinetobacter baumannii*, solvents, antibacterial activity.

INTRODUCTION

Humans are constantly exposed to potential harmful pathogens throughout their life which result in various diseases and have a great impact on their health. In the past a large number of chemical agents have been discovered or synthesized in order to treat and cure these infections; however, widespread and indiscriminate use of these drugs has led to the development of many drug resistant strains. These bacteria constitute a major problem worldwide as the existing drugs are becoming ineffective to control them. A big part of the world's population still relies on the benefits of food for the treatment of common illnesses [1]. Consequently, there is an urgent need to look for alternative of synthetic antibiotics and other alternate source of drugs. The possible solution for the aforesaid problems could come from spices and aromatic herbs which have been reported to have antioxidant and antimicrobial properties besides their numerous folk medicinal usage[2] [3].

Nutmeg is dried kernel of broadly ovoid seed of *Myristica fragrans* Houtt(Family: Myristicaceae). It is widely used as spices in culinary preparations and in alternative medicine as aphrodisiac[4], memory enhancer, antidiarrhoeal [5], anti-inflammatory and anticancer drug[6].Selection of Nutmeg for this study have been opted as their antimicrobial property has not been comprehensively evaluated. This contribution may be because of the presence of a variety of active phytochemicals like carotenoids, terpenoids, alkaloids flavonoids ligans and phenols in the plant. [7]. Medicinal plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics [8].

Gas Chromatography- Mass Spectroscopy, a hyphenated system is a very compatible and the most commonly used technique for identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [9].

MATERIALS AND METHODS

Collection of plant material

The fresh pericarp of *M. fragrans* fruits were collected from the Kulasekaram region of Kanyakumari District, TamilNadu. It was then cut into small pieces and dried for 7 days at room temperature (25°C). The dried samples were ground into fine powder and kept away from heat, moisture, and sunlight.

Preparation of plant extracts

Five hundred grams of dry powder of *M. fragrans* was sequentially extracted withsolvents such as toluene, tetrahydrofuran and methanol using the Soxhlet apparatus on the water bath for 12 h each. Each of the mixtures was carefully filtered using filter paper (Whatman No. 1) and concentrated using a rotary evaporator. The extracts were stored in sterile bottles at -18 ° C and kept as aliquots until further evaluation [10].

Phytochemical Analysis

The extracts were subjected to phytochemical screening for the presence of alkaloids, carbohydrates, tannins, terpenes, saponins, phenols, flavonoids, polysterols and proteins and amino acids as per standard methods [11].

Test organism

More than hundred sputum of suspected patients from various multispeciality hospitals of Kanyakumari district,TamilNadu were screened and sixteen isolates which were confirmed as the lower respiratory tract pathogen,*Acinetobacter baumannii* were used for the present study. The isolates were sub-cultured at 37 °C for 24 hours and maintained on nutrient agar slants. The pathogenic cultures were inoculated into sterile nutrient broth and incubated at 37°C for 3 hours. The pathogenic cultures were identified based on various morphological and biochemical tests using standard protocols [12] and the results were used for confirmation of the identity of the bacterial isolates according to Bergey's manual of determinative Bacteriology.

Preparation of Discs for Antibacterial Activity

Sterile Whatmann filter paper discs of size 10 mm, were soaked in the four solvent extracts of the test plant individually. These discs were then dried under controlled conditions of temperature to remove the excess of solvent and used for study.

Antibacterial Activity Using Disc Diffusion Method

The modified paper disc diffusion method was employed to determine the antibacterial activity of the plant extracts. The nutrient broth culture of *A. baumannii* was spread over the Muller Hinton agar plates using a sterile cotton swab in order to obtain

uniform microbial growth. Then the prepared antibacterial discs were gently kept over the lawn and pressed slightly along with positive and negative control. Meropenem 10 mcg/disc (Hi-Media) was used as positive control. The plates were incubated for 18-24 h at 37°C. The antibacterial activity was evaluated for 5 mg/disc and diameter of inhibition zones were measured [13].

Statistical Analysis

The statistical analysis of the antimicrobial activity of the seed extracts of the test plant was carried out as per standard methods [14].

Phytochemical screening

Phytochemical screening of the seed extracts of the test plant was carried out qualitatively for the presence of carbohydrates, terpenoids, tannins, flavonoids, phenolic compounds, saponins, phlobactanin, quinones and alkaloids [15]

GC MS Analysis

GC-MS technique was used in this study to identify the components present in the seed extract of the test plant. GC-MS technique was carried out at The *Cashew Export Promotion Council* (*CEPC*), Kollam, Kerala. GC-MS analysis was carried out on a GC clarus 500 Varian, USA system comprising a AOC-20I auto sampler and gas chromatograph interfaced to a mass spectrophotometer instrument employing the following conditions: Column Elite-1 fused silica capillary column (30mm×0.25mm I.D ×1 µ M df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 µ l was employed (split ratio of10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to 200 °C, then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time is 46min.

Result and Discussion

So far researchers have analysed essential oil, lignans and volatile aglycones from nutmeg and mace for its composition, antioxidant properties and antimicrobial activity [16] & [17]

The present study is an important report for presence of phytochemical and antimicrobial action of nutmeg using various solventextracts such as hexane, toluene, tetrahydrofuran, methanol and water. The selection of various solvents is based on the difference in their polarities. The main aim to choose different solvent is that maximum solubility of different constituents of nutmeg powder will depend upon the polarity of solvents. The objective of the study is to investigate antimicrobial activity against the dreadful lower respiratory tract and nosocomial pathogen *Acinetobacter baumannii* and also identification of phytochemical compounds for the activity by GC-MS. Toluene, tetrahydrofuran and methanol extracts of *Myristica fragrans* were tested for their antibacterial activity against *Acinetobacter baumannii* isolates. A good response was seen with methanol extracts of seeds of nutmeg showing the maximum inhibitory zone towards many of the isolates.

The highest zone of inhibition of 19mmwas recorded with the methanolic extract against the isolate A2 and the activity was above moderate against all the isolates.The anti bacterial activityof Toluene and tetrahydrofuran extracts was comparatively lower when compared to the methanol extract owing to their meagre extraction calibre (Table 2) The present study strongly supports that nutmeg fruit pericarp have strong antimicrobial activity against important pathogenic bacteria. Antimicrobial activity of different extracts could be attributed to the occurrence and concentration of various chemical substances present in that extract. Many compounds have been isolated from the nutmegs which are of antimicrobial importance.

Similar investigations with extracts of nutmeg were evaluated for antimicrobial activity against gram positive (*B. subtilis* and *S. aureus*), gram negative (*P. putida* and *P. aeruginosa*) bacteria and pathogenic fungi (*A. fumigatus*, *A. niger* and *A. flavus*) using disc diffusion method as well their MICs were reported earlier [18].

Phytochemical analysis of all extracts showed the presence of alkaloids, carbohydrates, flavonoids, terpenoids, phlobatanins and quinones, The absence of phenolic compounds and tannins and also the absence of Saponins except in the extraction with methanol was learnt.

Similar studies were carried out by the GC-MS analysis of rhizome of *Nervilia aragoana*, in which the analysis of the concentrated ethanol extract, ether extract, methanol extract revealed many compounds which have diverse use. Compounds having anti-inflammatory, antibacterial, antifungal, skin conditioning properties had been identified [19]

Around thirteen phytochemical compounds in methanolic extract of *Myristica fragrans* by GC-MS analysis were revealed in this study. The identified bioactive compounds possess many biological properties. The active principle Molecular Weight (MW), Molecular Formula (MF), and their Retention Time (RT) have been detected.(Fig 1 and Table 3).

This study explores the goodness of Nutmeg which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance. Some important antimicrobial compounds reported in nutmeg are α-pinene, β-pinene, p-cymene, β-caryophyllene and carvacrol [20] and [21]. Another important component for antimicrobial activities could be carvacrol. It has been reported that carvacrol can cross cell membranes and penetrate inside the cell where it interacts with intracellular sites critical for antimicrobial activities [22] and [23]. p-Cymene whose presence was disclosed in this study could also be important component because it is a precursor of carvacrol. It has been reported that p-cymene shows weak antibacterial activity but works synergistically with carvacrol in expanding the membrane which in turn causes destabilization of the bacterial membrane [24].Hence purification of the above compound and testing its antibacterial activity against the nosocomial lower respiratory tract pathogen and standardisation of the dose will be of great importance to the mankind.

CONCLUSION

Therefore, the seed *Myristica fragrans* extract possesses a significant inhibitory effect towards the potentially seious *A. baumannii* isolates. These results therefore support the traditional use of this plant product in pain and related conditions. However, further studies are necessary to examine the underlying mechanisms of the above mentioned phyto chemical constituents responsible for these pharmacological activities.

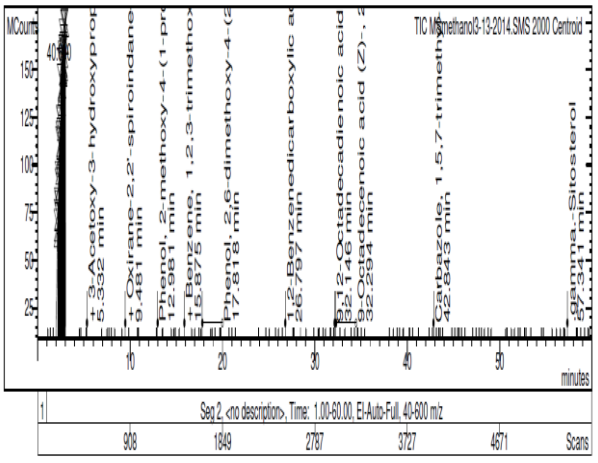


Fig. 1: Phytochemical compounds of the methanolic extract of *Myristica fragrans* using GC-MS analysis.

Table 1: Antimicrobial activities of *Myristica fragrans* against strains of *Acinetobacter baumannii*

Pathogen	Meropenem (mm)	MF 2 (mm)	MF 3 (mm)	MF 4 (mm)
A1	20	12	11	16
A2	16	10	13	19
A3	18	11	12	18
A4	24	10	9	14
A5	14	9	14	16
A6	17	10	10	13
A7	21	10	10	15
A8	19	14	7	18
A9	22	12	10	16
A10	16	11	12	14
A11	18	12	10	17
A12	21	12	10	15
A13	20	14	15	18
A14	23	12	11	14
A15	24	14	11	15
A16	19	10	11	14
SE D (+) -	1.8	1.1	1.2	1.4

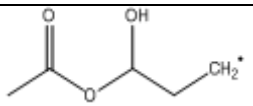
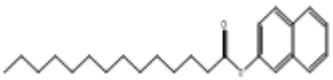
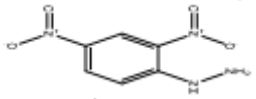
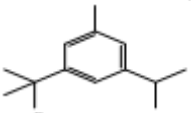
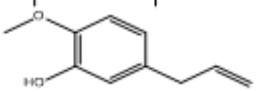
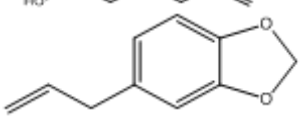
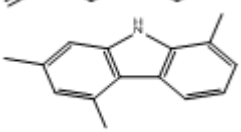
Key: (MF2) Toluene extract of *M.fragrans;* (MF3) Tetrahyrofuran extract of *M.fragrans;* (MF4) Methanolic extract of *M.fragrans;*

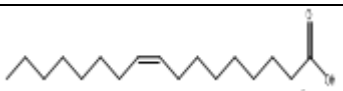
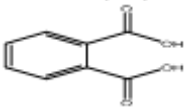
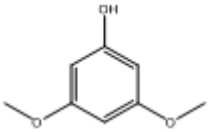
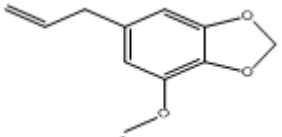
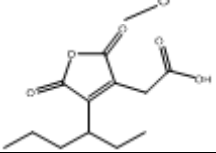
Table 2: Phytochemical analysis of *Myristica fragrans* using different solvents

Phytochemical Tests	Toluene	Tetrahydrofuran	Methanol
Alkaloids	+	+	+
Carbohydrates	+	+	+
Saponins	–	–	+
Phenolic compounds	–	–	–
Flavanoids	+	+	+
Terpenoids	+	+	+
Phlobatanins	+	+	+
Tannins	–	–	–
Quinones	+	+	+

Key : (+) Present; (-) Absent

Table: 3 Phytochemical analysis of seed extract of *Myristica fragrans* using GC-MS

S.No	Mol.Wt	Retention time	Formula	Compound Name	Structure
1	117.12	5.332	$C_5H_9O_3$	3-Acetoxy-3-hydroxypropyl	
2.	354.24	5.805	$C_{24}H_{34}O_2$	naphthalen-2-yl tetradecanoate	
3.	198.04	5.942	$C_6H_6N_4O_4$	(2,4-dinitrophenyl)hydrazine	
4.	190.17	7.584	$C_{14}H_{22}$	1-*tert*-butyl-3-isopropyl-5-methylbenzene	
5.	164.08	10.418	$C_{10}H_{12}O_2$	5-allyl-2-methoxyphenol	
6.	162.07	15.074	$C_{10}H_{10}O_2$	5-allylbenzo[*d*][1,3]dioxole	
7.	209.12	42.843	$C_{15}H_{15}N$	1,5,7-trimethyl-9*H*-carbazole	

8.	282.26	32.294	$C_{18}H_{34}O_2$	(*Z*)-heptadec-9-enoic acid	
9.	280.24	32.146	$C_{18}H_{32}O_2$	(9*E*,12*E*)-octadeca-9,12-dienoic acid	
10.	166.03	26.797	$C_8H_6O_4$	phthalic acid	
11.	154.06	12.981	$C_8H_{10}O_3$	3,5-dimethoxyphenol	
12.	192.08	15.074	$C_{11}H_{12}O_3$	6-allyl-4-methoxybenzo[*d*][1,3]dioxole	
13.	240.10	13.721	$C_{12}H_{16}O_5$	2-(4-(hexan-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl)acetic acid	

REFERENCES

1. Zhang X. Traditional Medicine: It is Importance and Protection. In: S. Twarog and P. Kapoor, (eds), "Protecting and Promoting Traditional Knowledge. Part 1, New York: United Nations; pp: 3-6; 2004.
2. Hinneburg, H.J.D. Dorman, R. Hiltunen. Food Chem., 97 (2006), pp. 122–129
3. Y. Yano, M. Satomi, H. Oikawa. Int. J. Food Microbiol., 111 (2006), pp. 6–11
4. Tajuddin, S. Ahmad, A. Latif, I.A. Qasmi, BMC Complement. Altern. Med., 3 (2003), pp. 6–10
5. J.K. Grover, S. Khandkar, V. Vats, Y. Dhunnoo, D. Das. Methods Find. Exp. Clin. Pharmacol., 24 (2002), pp. 675–680
6. O.A. Olajide, F.F. Ajayi, A.L. Ekhelar, S.O. Awe, J.M. Makinde, A.R.A. Alada, Phytother. Res., 13 (1999), pp. 344–345
7. K. M. Abdurrasheed, C. Janardanan, J. Spices Aromatic Crops, 18(2), 108 (2009).
8. Periyasamy A, Rajkumar, Mahalingam K., 2010. Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens. Middle-East Journal of Scientific Research, 5(6): 477-482
9. Ronald Hites A. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry, 1997. p. 609-611.
10. Sundar S K., Palavesam A and Parthipan B.,2008. Antimicrobial and phytochemical studies
11. of AM fungal treated selected medicinal plants of Kanyakumari District, Tamil nadu; College Sadhana Bloomers of Research; 1; 36-41.
12. Harborne J.B, 1998. Phytochemical methods:A guide to modern technique of plant analysis, Champman and Hall, London
13. Cuppucino and Shermann., 1996. Microbiology A Laboratory Manual; 6th Edition.
14. Bauer A.N., Kirby W.M.M., Sherries J.C and Truck M., 1996. Antibiotic susceptibility testing by a standardized single disk method. Amer.J.Clin.Pathol: 45(4): 493-436.
15. Gurumani. M J P Publications, An Introduction to Biostatistics. p-280, 2004
16. Harborne J.B, 1998. Phytochemical methods: A guide to modern technique of plant
17. analysis, Champman and Hall, London
18. Jukic.M. O. Politeo, M. Milos Croat. Chem. Acta, 79 (2006), pp. 209–214
19. Chatterjee.S Z. Niaz, S. Gautam, S. Adhikari, P.S. Variyar, A. Sharma Food Chem., 101 (2007), p. 515–523
20. Anish Deep Gupta, Vipin Kumar Bansal, Nishi Maithil.Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt).J. Genetic Eng.Biotech.Vol 11, (1), p 25-31
21. Elizabeth Thomas, Aneesh T., Della Grace Thomas, r. Anandan. GC-MS analysis of phytochemical compounds present in the rhizomes of *Nervilia aragoana* GAUD. Asian J Pharma Clinical Res. Vol 6, Suppl 3, 2013
22. H.J.D. Dorman, S.J. Deans J. Essent. Oil Res., 16 (2004), pp. 145–150
23. W.A. Konig, A. Kruger, D. Icheln, T. Runge J. High Resolut. Chromatogr., 15 (1992), pp. 184–189.
24. M. Cristani, M. D'Arrigo, G. Mandalari, F. Castelli, M.G. Sarpietro, D. Micieli, V. Venuti, G. Bisignano, A. Saija, D. Trombetta. J. Agric. Food Chem., 55 (2007), pp. 6300–6308
25. Ultee, E.P.W. Kets, E.J. Smid, Appl. Environ. Microbiol., 65 (1999), pp. 4606–4610
26. Ultee, M.H.J. Bennink, R. Moezelaar. Appl. Environ. Microbiol., 68 (2002), pp. 1561–1568