Biosciences, Biotechnology Research Asia Vol. **4**(2), 477-481 (2007)

Chemical constituents and bioactivity of steam distilled oil of *Monodora myristica* (Gaertn.) Dunal against some plant pathogenic fungi

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(Received: October 13, 2007; Accepted: November 22, 2007)

ABSTRACT

The chemical analysis of the essential oil of the dried seed nuts of *Monodora myrsitica* (Gaertn.) Dunal was investigated by GC and GC-MS. The results showed predominantly of hydrocarbon monoterpenes; p-cymene (31.5%), α-phellandrene (18.1%), α-pinene (6.1%), and β-pinene (5.1%)as major constituents. The bioactivity of the essential oil was evaluated against six plant pathogens by Micro broth dilution method. The essential oil suppressed the growth of these plant pathogenic fungi in culture and shows high to low significant potency.

Keywords: *Monodora myrsitica,* essential oil chemical analysis, hydrocarbon monoterpenes, Micro broth dilution method, plant pathogens, fungi.

INTRODUCTION

Monodora myristica (Gaertn.) Dunal is known as *Calabash* Nutmeg, African or Jamaican nutmeg, belongs to the family Annonaceae, a tropical to subtropical plant family that is widely distributed from Liberia, in West Africa to Cameroon, Central Africa, Asia, Central and South America and Australia (Ekundayo, 1989). The seeds are sold in the markets in rural areas all over West Africa and are used as condiment in soup and stimulating addition to other medicines and snuff (Iwu, 1993). *M. myristica* oil are used whole for flavouring food products and to cure various gastro-intestinal complaints, psychological disorders and urinary diseases (Usmanghani *et al.,* 1997 and Adegoke *et al.,* 1998). The volatile constituents of *M. myrsitica* has been extensively studied (Ikedia *et al.,* 1962; Ekundayo and Hammerschmidt, 1988; Onyenekwe *et al.,* 1992; Olajide *et al.,* 1999; Simpson and Jackson, 2002 and Olawore, *et al.,* 2002). Report have it that the main constituents established have been α-phellandrene, α-pinene, β-pinene and terpinen-4-ol. There are considerable variations in the content of the major components within the species. Because of the significant difference in geographical location (Lawrence, 1988) culture and environmental conditions (Charles and Simon, 1990) and different chemotypes in the oil composition, we have focused this study to investigate the chemical constituents responsible for the characteristic odour and bioactivity of steam distilled oil of *M. myrsitica* against some plant pathogenic fungi from Lagos in comparison with the one from Imo state as reported by (Onyenekwe *et al.,* 1992) and the one from Oyo state all in Nigeria as reported by(Olawore *et al.,* 2002).

MATERIAL AND METHODS

Plant materials

The dried seed nuts of *M. myristica* were obtained from a local market in Lagos, Nigeria in August 2006. The nuts were identified by Mr. O.K. Oluwa from the Botany Department, Lagos State University, where a specimen was deposited. The dried seed nuts (350g) were crushed into coarsed in a grinder and subjected to hydrodistillation for 4hrs in an all glass Clevenger-type apparatus (British Phamacopoeia, 1980). The oil obtained was dried over anhydrous sodium sulphate and stored in the refrigerator until it was sent to DDU Gorakhpur University, Gorakhpur, India, (Chemistry Department) for analysis.

Identification of the essential oil components

The volatile constituents were analysed by gas chromatography coupled to mass spectrometry (GC-MS). GC-MS analysis was carried out on a Hewlett -Packard HP 6890 equipped with Hewlett Packard mass Detector (model 5973) and a HP-5 Column 30m x 0.25mm i.d (cross linked 5% phenylmethylsiloxane). The injector, GC-MS inter phase, ion source and selected mass detector temperatures were maintained at 270°C, 280°C and 150°C respectively. Helium was used at the carrier gas and the oven temperature was programmed as follows: 60°C for 5 mins, rising at 1°C/min to 140°C, then at 10°C/min to 270°C and held for 5min. Chemical components of the oil were identified by comparing their mass spectra and retention indices ((RI) with those of standard included in the library NBS 75k (Adams, 1989) and the results were reported in Table 1.

Antifungal Activity

The micro-organisms screening were performed according to (Smith *et al.,* 2002) using certain fungi pathogens obtained from Microbiology Department, Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos, Nigeria. *Fusarium sambucinum, Rhizoctania solani, Aternaria solani Aspergillus niger, pencillium sclerotigenum and Rhizopus nodosus.* Micro broth dilution method (Smith *et al.,* 2002) with slight modification was performed as follows. The fungi were cultured on Potato dextrose agar (PDA) medium at 28°C for one

Table 1: Chemical constituents of the essential oil of *Monodora myristica* seeds

Peak	Compound	RI[a]	%
1.	α-Thujene	928	3.4
2.	α-Pinene	932	6.1
3.	4-Carene	958	0.1
4.	Sabinene	973	0.4
5.	α-Pinene	976	5.1
6.	2-Carene	1001	1.0
7.	α-Phellandrene	1009	**18.1**
8.	3-Carene	1011	0.05
9	α-Terpinene	1017	0.1
10.	*p*-Cymene	1030	**31.5**
11.	(E)-β-Ocimene	1039	0.5
12.	(Z)-β-Ocimene	1048	0.2
13.	γ-Terpinene	1059	0.1
14.	Terpinolene	1087	0.2
15.	Linalool	1102	4.2
16.	β-Thujone	1116	0.02
17.	2,8-Mentadien-1-ol	1121	0.7
18.	α-Campholenal	1125	0.02
19.	2-Methen-1-ol	1138	0.6
20.	*cis*-Verbenol	1144	0.2
21.	*trans*-2-caren-4-ol	1154	0.1
22.	Borneol	1165	0.3
23.	Naphthalene	1178	0.3
24.	α-Terpineol	1190	1.4
25.	*cis*-Piperitol	1194	Trace
26.	*cis*-Sabinol	1206	8.9
27.	*Trans*-Piperitol	1210	0.5
28.	5-Acetoxy-2-furaldehyde	1223	0.6
29.	Carvatanacetone	1246	0.1
30.	Piperitone	1253	0.2
31.	Geraniol	1256	0.2
32.	Carvacrol	1306	2.0
33.	α-Terpenyl propionate	1348	0.1
34.	Eugenol	1357	0.6
35.	α-Copaene	1372	0.2
36.	Geranyl acetate	1385	0.3
37.	(E)-β-Caryophyllene	1414	0.4
38.	α-Humulene	1448	0.3
39.	γ-Muurolene	1473	0.2
40.	D-Germacrene	1476	0.1
41.	ar-Curcumene	1481	0.05
42.	*epi*-Cubebol	1491	0.4
43.	α-Zingiberene	1494	0.05
44.	α-Muurolene	1497	0.4
45.	α-Cadinene	1510	1.1
46.	Cubebol	1512	0.2
47.	δ-Cadinene	1520	1.6
48.	Germacrene D-4-ol	1570	1.1
49.	1,10-di-*epi*-Cubenol	1610	0.5
50.	δ-Cadinol	1643	0.05
51.	α-Cadinol	1651	0.4
	Total		**95.24**

HMT 66.55 OMT 23.79 HST 4.9

[a] Elution order and retention indices on a HP-5 Column (see Experimental) compared with authentic sample

week before the experiment. The Nutrient agar medium was prepared and distributed on sterile Petri dishes (80 mm diameter) which were aseptically placed in a chamber without lid. The essential oil was emulsified at the ratio 1:9 (v/v) in 0.1% water agar and separately introduced into each of the Petri dish and tightly covered. Incubation in the Petri dish was performed at 28°C for 3 to 5 days the period for colony formation. Control experiment was set up without the addition of essential oil and the period required for colony formation on the control Petri dish was determined. For each, three replicates were carried out and the average was determined. The fungi toxicity was determined by measuring the diameter of the zone of inhibition and its percentage colony inhibition was calculated according to the formula of (Pandey *et al.,* 1982) with reference to the negative control. The results of the antifungal activity were shown in Table 2.

Growth inhibition (%) = {(C - T)/ C} x 100
Where C = average zone inhibition of control, and
T = average zone inhibition of fungal colony with essential oil.

Table 2: Antifungal activity of the essential oil of *Mondora myristica* found in Lagos, Nigeria

Micro-organism	Acitvity	Growth inhibition(mm)	Growth inhibiton %
Fusarium sambucinum	++	9	47.4
Rhizoctania solani	+	3	15.8
Alternaria solani	++	8	42.1
Aspergillus niger	+++	12	63.2
Pencillium sclerotigenum	+++	13	68.4
Rhizopus nodosus	+++	12	63.2

+++strongly activity; ++ moderately activity; + weak activity.
The results are the average of three readings

RESULTS AND DISCUSSION

The obtained essential oil after 4hrs of hydrodistillation yielded 1.4 % (v/w). Table 1 shows the constituents identified by GC-MS in the oil together with their Retention indices and percentage compositions. Quantitatively, the oil was characterized by high amount of hydrocarbon monoterpenes (HMT) (66.55%). The predominant hydrocarbon monoterpenes were *p-cymene* (31.5%), *α-phellandrene* (18.1%), *α-pinene* (6.1%), *α-pinene* (5.1%), *β-Thujene* (3.4%). Hydrocarbon sesquiterpenes (HST) (4.9%) were also detected. The detection of *p-cymene* and *α-phellandrene* as the most abundant constituents makes it similar to an earlier report (Ekundayo and Hammerschmidt 1988 and Olawore *et al.,* 2002). Furthermore, oxygenated monoterpenes were also identified comprising of eighteen alcohols (22.35%), two aldehydes (0.62%) three ketones (0.32%) and three esters (0.5%). The result showed that a number of other constituents which were not reported by (Ekundayo and Hammerschmidt 1988; Onyenekwe *et al.,* 1992 and Olawore *et al.,* 2002) were also identified. Some of these constituents included *cis sabinol* (8.9%), *α-pinene* (5.1%), *carvacrol* (2.0%), 2*-carene* (1.0%), *eugenol* (0.6%), (E)-*β-ocimene* (0.5%), *epi-cubebol* (0.4%) and *α–cadinol* (0.4%). This result showed that there were variations in the chemical constituents of this plant; some constituents were present as found in Imo and Oyo States and not found in Lagos State vice versa, these variations could probably be due to geographical origin (Lawrence, 1988), genetic factors, culture and environmental conditions (Charles and Simon, 1990), different chemotypes, and nutritional status of the plant as well as other factors that can influence the oil constituent. The reported concentration of *p-cymene, α-pinene, linalool* and *α–thujene* in the oil *M.myristica* is in conformity with previous work on nut meg (Ikedia *et al*., 1962; Baldry *et al.,* 1976 and Olawore *et al.,* 2002).

The essential oil of *M. myrsitica* exhibited strong biological activity against tested pathogens. The highest activities were obtained with *Pencillium sclerotigenum, Aspergillus niger* and *Rhizopus nodosus.* Also, the oil was moderately effective against *Alternaria solani* and *Fusarium sambucinum* while slightly potent promise was observed against *Rhizoctania solani*. The variation of the biological activity could be correlated to chemical composition variability (Burt, 2004 and Lahlon, 2004). The control experiment showed uninhibited growth of the plant pathogens. It has also been reported that some volatile components from aromatic plants especially essential oil of *M. myrsitica* have been demonstrated to possess high antimicrobial activity (Dorman and Deans 2002; Tatsadjieu *et al.,* 2003; Nguefack *et al.,* 2004 and Odoh *et al.,* 2004) and insecticidal (Okonkwo and Okoye, 1996) properties. The interactions between essential oil components play an important role in the determination of their biological activities.

Studies have shown that high concentration of *p-cymene* in the oil makes it potentially useful in the cream and food industries (Ultee *et al.,* 2000 and Nguefack *et al.,* 2004), and could possibly be applied as food preservatives by rural dwellers that frown at the use of synthetic chemicals that are hazardous to the health. The study of the stability of the bioactivity of the essential oil showed enhancement of storage life up to 5 days. In order to achieve a longer preservation using this oil, it may be necessary to consider reapplication after an expiration of 5 days of the first use, since the activity of essential oils decreases with time because of their high volatility. Therefore the oil could be recommended as a potential source of eco-friendly botanical fungicide, after long term and wide ranging trials.

ACKNOWLEDGMENTS

The authors are thankful to Dr. A.A Kasali, Dr. I.A Ogunwande and Mr A. Eshinlokun, Chemistry department Lagos State University for their technical assistance. Also we grateful to Mr. S.A. Ajani, Microbiology Department, Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria for screening the plant pathogens.

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