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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRIC ANALYSIS OF THE CHEMICAL CONSTITUENTS FROM CHLOROFORM FRACTION OF *MONODORA MYRISTICA* METHANOL SEED EXTRACT

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ABSTRACT
Monodora myristica is a popular spice sold in most west african markets. The seeds are used in the preparation of soups and stews. The seed extract is used for the treatment of headaches, sores and stomach upsets. It is also used as an insecticide. The aim of this research was to identify the phytochemicals present in the *Monodora myristica* seed cake. Extraction was carried out with both polar and non polar solvents. The polar extract was fractionated with solvents of increasing polarities. Phytochemical screening was carried out on both crude extracts and the fractions obtained from fractionation of the methanolic crude extract. The Chloroform fraction was subjected to gas chromatogaphy-mass spectrometric analysis. Phytochemical screening of the crude extracts of the seed cake revealed presence of steroids and terpenes in the n-hexane and chloroform extracts while saponins, flavonoids, tannins and glycosides were present in the ethyl acetate, methanol and water extracts. No alkaloid was detected in all the extracts. The fractions obtained from fractionation of the crude methanolic extract showed presence of glycosides and saponins in the n-hexane fraction, tannins, saponins and flavonoids in the ethyl acetate fraction while the n-butanol fraction had just tannins in it. The chloroform fraction of the methanolic extract showed presence of terpenoids, flavonoids and phenolics. The GC-MS analysis of the chlorofrom fraction revealed presence of 32 compounds but only 27 of these were identified. Most of the identified compounds belong to the class of phenolics, terpenoids and fatty acids. Some of the identified compounds possess some biological properties. Monodora myristica seed possess phytochemicals which could be useful in pharmceutical industry.

Keywords: *Monodora Myristica, Seed, Phytochemical Screening, Fractionation, Extract, GC-MS*

INTRODUCTION
Spices are used by most cultures to enhance flavour and aroma. Some of these spices possess medicinal properties and therefore, should not be overlooked. Researchers have shown that some of the phytochemicals present in these spices possess antioxidant, anti-inflammatory, antimicrobial, etc, properties. Knowledge of these phytochemicals is important because they can serve as new source of drugs for the treatment of sickness and diseases which are resistant to synthetic drugs. One of such spices is *Monodora mtristica.* It is a tree that grows in evergreen forests from Liberia to Nigeria and Cameroon to Kenya (Koudou *et al*., 2007; Aganaiet and Bessiere, 2004). The *Monodora myristica* tree can reach a height of 35 m and 2 m in diameter. It has a clear trunk and branches horizontally. The leaves are alternately arranged and drooping with the leaf blade being elliptical, oblong or broadest towards the apex and tapering to the stalk. They are petiolate and can reach a size of up to 45 x 20 cm. The flower appears at the base of new shoots and is singular, pendant, large and fragrant. The pedicel bears a leaf-like bract and can reach 20 cm in length. The flower's sepals are red-spotted, crisped and 2.5 cm long. The corolla is formed of six petals of which the three outer reach a length of 10 cm and show curled margins and red, green and yellow spots. The three inner petals are almost triangular and form a white-yellowish cone which on the outside is red-spotted and green on the inside. The flower's stigmas become receptive before its stamens mature and shed their pollen (protogynous). The flower is pollinated by insects. The fruit is a berry of 20 cm diameter and is smooth, green and spherical and becomes woody. It is attached to a long stalk which is up to 60 cm long. Inside the fruit the numerous oblongoid, pale brown, 1.5 cm long seeds

are surrounded by a whitish fragrant pulp. The fruits are collected from wild trees and the seeds are dried and sold whole or ground to be used in stews, soups, cakes and desserts (Koudou *et al.*, 2007). The odour and taste of the *Monodora myristica* seed is similar to nutmeg and it is used as a popular spice in the West African cuisine (Aganaiet and Bessiere, 2004). For medicinal purposes they are used as stimulants, stomachic, for headaches, sores and also as insect repellent. The bark is used for the treatment of stomach-aches, febrile pains, eye diseases and haemorrhoids (Cimanga *et al*., 2002). The essential oil that can be obtained from the leaves contains β-caryophyllene, α-humulene and α-pinene. The major compounds found in the essential oil from the seeds are α-phellandrene, α-pinene, myrcene, limonene and pinene (Nguatack *et al*., 2004; Oussou *et al*., 2004; Tatsadjieu *et al*., 2003). The aim of this research work is to determine other phyto-components that are present in the seed cake of *Monodora myristica* using GC-MS as our analytical tool.

MATERIALS AND METHODS

Sample Collection and Preparation

All chemicals used were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

The *Monodora myristica* (African nutmeg) seeds were obtained from a traditional health practitioner at Oja Jagun, Ogbomoso, Oyo State, Nigeria. The seeds were identified by Mrs. A. F. Ogundola of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The seeds were dehosed and pulverized into fine powder using a sterilized food mill.

Preparation of Extract

200 g of grinded *Monodora myristica* seeds were soaked in methanol for 72 hours. It was filtered and the filtrate concentrated. All traces of solvent were removed by evaporation. After evaporation the methanolic extract (7.33%) appeared as dark brown powder. It was suspended in water (200 ml) and sequentially extracted using the solvents n-hexane, chloroform and n-butanol (3 x 200 ml).

Phytochemical Screening

Phytochemical screening was carried out according to the method described by Harborne (1973). The extracts and fractions obtained from fractionation of the crude methanolic extract were screened for the presence of alkaloids, flavonoids, saponins, steroids, tannins and glycosides.

Experimental Procedure for GC-MS

The chloroform fraction was subjected to GC-MS.

Gas Chromatographic Programme	
Equipment	Agilent 5975C inert MSD with triple axis detector
Column	Agilent 19091S-433HP-5MS (30 m x 250 µm x 0.25 µm 5% phenyl methylsiloxane)
Carrier gas	Helium (constant flow rate 1.5 ml/min)
Sample injected	1µl
Injection temperature	240ºC
Oven temperature	100ºC
Transfer temperature	300ºC
Total GC running time	49 min
Mode	Split
Split ratio	50:1
Run time	49 min
Mass Spectrometric Programme	
Inlet line temperature	200ºC
Source temperature	250ºC
Electron energy	70eV
Mass scan (m/z)	50 - 600 amu
Solvent delay	5.0 min
Library	NIST version year -2011

RESULTS AND DISCUSSION

The knowledge of chemical constituents present in plants is very important. This can add to available database of medicinal plants. Such knowledge can lead to the discovery of drug candidates and drug precursors which can be modified by the application of chemical synthesis. The preliminary phytochemical screening of the crude extracts revealed presence of steroids and terpenes in the n-hexane and chloroform extracts while saponins, flavonoids, tannins and glycosides were present in the ethyl acetate, methanol and water extracts. Alkaloids were absent in all the extracts. The phytochemical screening of the fractions obtained from fractionation of the crude methanolic extract showed presence of glycosides and saponins in the n-hexane fraction, tannins, saponins and flavonoids in the ethyl acetate fraction while the n-butanol fraction had just tannins in it. The chloroform fraction of the methanolic extract showed presence of terpenoids, flavonoids and phenolics. Researchers have shown that plant phenolics have important medicinal properties such as antioxidative and their use as cellular support materials (Gupta *et al.,* 2010). Other biological properties are antiapoptosis, anti-inflammatory, anticancinogenic, antiartherosclerosis, inhibition of angiogenesis, improvement of endothelial function, cell proliferation (Hans *et al.,* 2007) and disinfectant (Okwu, 2001). Terpenoids are used for flavouring and perfumery. The GC-MS analysis of the chloroform fraction obtained from fractionation of the methanolic seed extract cake of *Monodora myristica* revealed presence of thirty two compounds as obtained in the chromatogram shown in figure 1. Out these thirty two compounds only five were unidentified. The twenty seven compounds were identified based on their retention time, molecular formula, molecular weight and peak area in percentage. The interpretation of the mass spectrum of each compound was based on comparison of the mass spectrum of the unknown compound with those of NIST-2011 (National Institute of Standards and Technology) library stored in the computer database of the GC-MS equipment. The first compound to emerge from the GC analysis was o-cymene which is a terpenoid with retention time of 15.802 min while the last to emerge was Butyl-9-octadecenoate which is an ester with retention time of 42.957 min. The most abundant compound in the chloroform fraction was identified as cis-vacennic acid (17.166%). All the identified compounds are given in Table 1. The GC-MS analysis revealed that most of the identified compounds were terpenoids, fatty acids and fatty acid esters. The biological importance of some of the identified compounds has been reported. N-hexadecanoic acid possesses antioxidant, anti-inflammatory, hypocholesterolenic, nematicide, pesticide, antiandrogenic and hemolytic properties. It is also a 5-α reductase inhibitor (Gomathy *et al.,* 2012). Quercetin has been reported to exhibit both immunosuppressive and immunomodolatory properties (Gong and Chen, 2003; Kim *et al.,* 2005; Min *et al.,* 2007; Morcira *et al.,* 2007; Huang *et al.,* 2010; Yu *et al.,* 2010). O-cymene is used both as a flavouring agent and as a fragrance in the Food and Fragrance industries. Thymol is used as an antiseptic, a herbal supplement, and for the treatment of bronchitis (drugs.com).

Table 1: Phytochemical Screening of Crude Extracts of *Monodora Myristica* Seed

Extracts	Steroids	Saponin	Alkaloids	Flavonoids	Tannins	Glycosides	Terpenoids
n-hexane	+	-	-	-	-	+	-
Chloroform	+	-	-	-	-	+	-
Ethyl acetate	-	+	-	+	+	+	-
Methanol	-	+	-	+	+	+	+
Water	-	+	-	+	+	+	-

Table 2: Phytochemical Screening of Methanolic Fractions of *Monodora Myristica* Seed

Extracts	Steroids	Saponin	Alkaloids	Flavonoids	Tannins	Glycosides	Terpenoids
n-hexane	-	+	-	-	-	+	-
Chloroform	-	-	-	+	-	+	+
Ethyl acetate	-	+	-	+	+	+	-
n-butanol	-	+	-	+	+	+	-

Table 3: GC-MS Analytical Report for the Chloroform Fraction

Peak Number	Retention Time (Min)	Peak Area	Molecular Mass	Molecular Formula	Identified Compound
1	15.802	18893567	134	$C_{10}H_{16}O$	o-Cymene
2	21.999	10461713	152	$C_{10}H_{16}O$	Trans-2-caren-4-ol
3	22. 918	18336086	152	$C_{10}H_{16}O$	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1α, 3α, 5α)
4	26.375	9296045	150	$C_{10}H_{14}O$	Thymol
5	26.917	40454530	150	$C_{11}H_{18}$	Cyclohexene, 2-ethenyl-1, 3, 3-trimethyl-
6	27.506	123395000	137	$C_7H_7NO_2$	Carbamic acid, phenyl ester
7	27.797	14708724	155	-	-
8	29.674	19099400	169	-	-
9	30.742	84978518	170	$C_{10}H_{18}O_2$	1, 3, 3-trimethyl-2-oxobicyclo[2.2.2]octan-6-ol
10	31.088	26973368	168	$C_{10}H_{16}O_2$	2-Cyclohexen-1-one, 4- hydroxy-3-methyl-6-(1- methylethyl)-, trans
11	31.756	32470443	168	$C_{10}H_{16}O_2$	2-Cyclohexen-1-one, 4- hydroxy-3-methyl-6-(1- methylethyl)-, cis
12	33.138	27555086	206	$C_{14}H_{22}O$	4-(2,2-dimethyl-6-methylenecyclohexylidene)-3-methyl-2-butanone
13	33.390	13564344	206	$C_{14}H_{22}O$	Phenol, 2, 4-bis(1, 1-dimethyl)-
14	37.247	5564195	222	$C_{15}H_{25}O$	α-cadinol
15	37.443	5902982	222	$C_{15}H_{25}O$	1, 4-methanoazulen-3-ol, decahydro-1, 5, 5, 8a-tetramethy-, [1S-(1α, 3β, 3αβ, 4α, 8αβ)]-

16	38.056	23289669	280	$C_{16}H_{24}O_4$	Acetic acid, 2, 6, 6-trimethyl-3-methylene-7-(3-oxobutylidene)oxepan-2-yl ester
17	38.386	5271875	237	-	-
18	38.511	125185992	238	$C_{16}H_{30}O$	cis-9-hexadecenal
19	39.265	30314222	220		(1, 1-bicyclohexyl)-2-ol, 5-(1, 1-dimethylethyl)-
20	39.399	74972416	220	$C_{13}H_{22}O$	6-isopropenyl-4, 8a-dimethyl-1, 2, 3, 5, 6, 7, 8, 8a-octahydro-naphthalen-2-ol
21	39.768	6367615	281	$C_{19}H_{23}NO$	9-octadecamide
22	40.090	5319360	-	-	-
23	40.287	7789194	297	-	-
24	40.389	25231184	256	$C_{16}H_{32}O_2$	n-hexadecanoic acid
25	40.695	5542647	355	$C_{21}H_{38}O_4$	9, 12-octadecadienoic acid (Z, Z)-2, 3-dihydroxypropyl ester
26	40.821	14854317	241	$C_{15}H_{15}NO_2$	(2, 2, 6-trimethyl-5, 6-dihydro-2H-pyrano[3, 2-c]quinoline-5-one
27	40.946	5508558	302	$C_{15}H_{10}O_7$	Quercetin
28	41.064	12473837	294	$C_{19}H_{28}O_2$	9,12-octadecadienoic acid, methyl ester, (E, E)-
29	41.096	12010269	296	$C_{19}H_{36}O_2$	10-octadecenoic acid, methyl ester
30	41.551	171968490	282	$C_{18}H_{34}O_2$	Cis-vaccenic acid
31	42.769	8200706	400	$C_{28}H_{48}O$	Campestrol
32	42.957	15849396	339	$C_{22}H_{40}O_2$	Butyl-9-octadecenoate

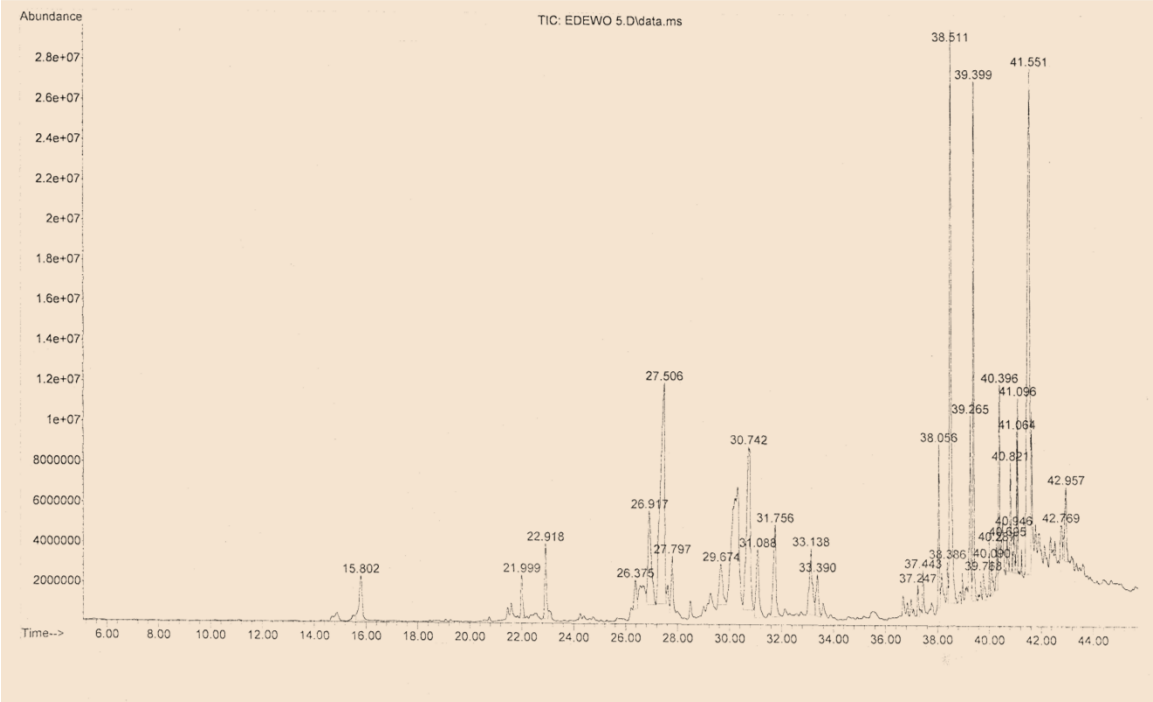

Figure 1: Total Ion Chromatogram of the Chloroform Fraction

Conclusion

The identified compounds confirm the use of the seed as a stimulant, insecticide and flavouring agent. Further work is on-going to isolate, characterize and determine the biological parameters of the compounds. The identified compounds could serve as important lead compounds in the synthesis of potent drugs and chemicals which can find use in the pharmaceutical and flavouring industries.

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