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Research Article
Characterisation of Phytochemicals in Raw and Processed *Monodora myristica* (Gaertn.) Dunal Seeds by UPLC-MS

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Abstract

Background and Objective: *Monodora myristica* (*M. myristica*) seeds are processed locally using various indigenous knowledge systems (IKS) based processing techniques like boiling, roasting and frying for varying lengths of time. It is important to determine the effect of these processing methods on its phytochemical constituents, a step necessary in order to explain its nutritional/medicinal use. This paper determined the effects of different cooking methods (boiling and roasting) and cooking times (10, 20 and 30 min) on the phytochemical constituents of *M. myristica* seeds using ultra performance liquid chromatograph-mass spectrometer (UPLC-MS). **Materials and Methods:** *M. myristica* seeds were thermally processed through boiling and roasting for varying lengths of time. Metabolite profiling using UPLC-MS was utilized to identify phytochemicals in raw and processed seeds. Metabolites were characterized by their UV-vis spectra, retention times relative to external standards, mass spectra and comparison to in-house database, phytochemical dictionary of natural products database and reference literature. **Results:** A total of 32 metabolites were identified, including terpenoids, sterols, alkaloids, fatty acids, saponins, flavonoids, glycosides and coumarins. Processing induced changes in phytochemical composition, more phytochemicals were identified in the roasted samples and the raw (control) sample had the least (four) number of phytochemicals. **Conclusion:** These findings are promising as they indicate that suitable processing techniques could be established and then applied in the development of new functional foods from whole *M. myristica* seeds or its extracts. *M. myristica* seed can be considered a good source of phytochemicals.

Key words: Spice, phytochemicals, UPLC-MS, *Monodora myristica*, thermal processing

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Monodora myristica is a perennial edible plant of the Annonaceae or custard apple family of flowering plants[1,2]. The seed is a popular spice used in cooking to add flavour and thicken dishes[3]. It is variously known as *Iwor* amongst the Itsekiris, *Ikposa* (Benin), *Ehiri* or *Ehuru* (Ibo), *Gujiya dan miya* (Hausa) and *Ariwo, arigbo, Abo lakoshe* or *eyi naghose* (Yoruba), *Ehinawosin* (Ikale), *Uyengben* (Edo), *Fausse noix de muscade* (French)[4-6]. Studies have shown that almost every part of the tree is important economically and a number of medicinal properties have been ascribed to various parts of this highly esteemed plant[7]. The most economically important parts are the seeds[8]. The presence of bioactive compounds in the plant makes it possible for the seeds to be used in traditional medicine as well as a spice in local foods[9]. The aromatic seeds are antiemetic, astringent, anti-inflammatory, antipyretic, aperient, stimulant, stomachic, tonic and they are added to medicines to impart stimulating properties[8,10,11].

The presence of bioactives in edible plants is largely influenced by several factors such as genetic factors, environmental conditions, degree of ripening, variety, processing techniques and storage methods[12]. *Monodora myristica* seeds are processed locally (in the sub-Sahara African regions) using various indigenous knowledge systems (IKS) based processing techniques like boiling, roasting and frying for varying lengths of time. They are then dehulled and crushed into flour for use in local dishes, such as the West African "kunu," "tuwo," and "waina". Some natives simply dehull using stone and crush the raw seed for use in local dishes.

Feyisayo and Oluokun[13] identified phenolics present in the seeds of *M. myristica* by gas chromatography (GC) coupled to flame ionization detector (FID). They estimated the total phenolic content as 1478.32 mg/100 g and concluded that *M. myristica* is a good source of phenolics. Other reports on the phytochemical screening of the seeds of this plant revealed the presence of phenolic compounds, including flavonoids and tannins, cyanogenic glycosides, alkaloids, arocine, lactose, terpene, resins, fiberro-latic oils, anthraquinones, saponins, , steroids, oxalates and phytates[14,15]. Processing of plants for consumption may cause chemical reactions that result in changes in phytochemical constituents and hence health-benefitting properties of the plant[16]. A comprehensive review of the literature showed that there is no report on the effect of processing methods on the metabolite composition of *M. myristica* seed flour. Due to their overwhelming advantages, UPLC-MS based methods are a suitable tool for the investigation of natural products in food[17,18]. The current study was conducted to characterize the secondary metabolites present in raw and processed *M. myristica* seeds using UPLC-MS instrument equipped with time of flight (TOF) detectors.

MATERIALS AND METHODS

Reagents and chemicals: Unless otherwise stated, all the chemicals/reagents used were of analytical grade from Sigma-Aldrich Co., Ltd (Steinheim, Germany). Water was purified by using a Milli-Q system (Millipore, Bedford, USA).

Sample: *Monodora myristica* seeds were purchased from a local market, in Ado-Ekiti, Ekiti State, Nigeria.

Sample preparation: The seeds were cleaned and extraneous materials like dry leaves and stones removed. Samples were divided into seven portions and prepared using the method of Mbah *et al.*[19] with slight modification. The first portion was raw and it served as the control. The second, third and fourth portions were boiled (100°C) in a pot of tap water in a ratio of 1:3 (weights of the seeds to volume of water) for varying times: 10, 20 and 30 min. After boiling, the seeds were oven dried at 100°C for 5 h, dehulled and milled into fine flour. The remaining fifth, sixth and seventh portions were roasted (120°C) for different times (10, 20 and 30 min), dehulled and milled into fine flour. The control seeds were dehulled and milled without any thermal processing. Flour samples were defatted for 4 h using a Buchi 810 Soxhlet Fat Extractor (Flawil, Switzerland) and packaged in labelled polythene bags (CO: raw flour, B10, B20, B30: flour from seeds boiled for 10, 20 and 30 min, respectively, R10, R20, R30: flour samples from seeds roasted for 10, 20 and 30 min, respectively). The packaged flour samples were stored in a cool (±4°C) dry place until required for analysis.

Preparation of methanolic extracts: Flour samples were extracted (1:5 w/v) exhaustively with methanol. The extracts separately were concentrated to dryness under reduced pressure in a rotary evaporator (40°C). Dried extracts were re-dissolved in methanol for further experiments.

Ultra-high performance liquid chromatography mass spectrometry (UPLC-MS) analysis: First, 50 mg of each dried sample was re-dissolved in 5 mL LC-MS grade methanol. The sample solutions were then prepared to a concentration of 200 ppm in methanol and filtered into analysis vial using a filter membrane. The samples were analysed with a UPLC-MS, which consisted of mass spectrometer (Waters Corporation,

Micromass MS Technologies, Manchester, UK) coupled to an ultra high performance liquid chromatography System (Alliance 2695 UPLC system, Waters Corporation, Milford, MA, USA). The mass spectrometer was equipped with a UV-VIS and time of flight (TOF) detectors, whose accurate mass measurements provide direct reliable information on molecular formulas. Compounds were separated on an Atlantis T3 C18 column (Waters Corporation, Milford, USA, 100 mm×2.1 mm, 3 µm particle size) using 0.5% aqueous formic acid (solvent A) and 0.5% formic acid in 50/50 v/v acetonitrile:methanol (solvent B). Column temperature was maintained at 40°C. A stepwise gradient from 10-90% solvent B was applied at a flow rate of 0.2 mL min^{-1} for 26 min. Mass spectra data were recorded for a mass range m/z 100-m/z 1000. Capillary voltage and cone voltage were set at 3 kV and 30 V, respectively. In all searches for elemental composition, 0-100 atoms of carbon, 0-100 atoms of hydrogen, 0-50 atoms of oxygen and 0-50 atoms of nitrogen were included. Experimental and theoretical mass were separated by an error not larger than 5 ppm. Mass spectral data was processed using OpenLynx (Waters Corporation, Milford, MA, USA).

RESULTS AND DISCUSSION

A total of 32 metabolites were identified including triterpenes, alkaloids, saponins, flavonoids and coumarins. Metabolites were identified by their UV-vis spectra, retention times relative to external standards, mass spectra and comparison to in-house database, phytochemical dictionary of natural products database and reference literature. Alkaloids accounted for the highest abundance in the samples (Table 1). Alkaloids are a heterogeneous class of secondary metabolites traditionally defined as basic (alkali like), nitrogen containing organic constituents that occur mainly in plants[20]. Alkaloids and extracts of alkaloid containing plants often have

Table 1: Metabolites from the methanolic extracts of raw and processed *Monodora myristica* seeds

No.	Compounds	Molecular formula	m/z Experimental	m/z Calculated	Error (ppm)	RT (min)	Detected in
1	Ixoside	$C_{16}H_{19}O_{11}$	387.0934	387.0927	1.8	1.24	CO, R10, R20, B30
2	(-)-Epicatechin-3-(3''-O-methyl) gallate	$C_{23}H_{19}O_{10}$	455.0956	455.0978	-4.8	1.26	B10
3	Oxiamycin	$C_{23}H_{24}NO_4$	378.1700	378.1705	-1.3	8.59	R30
4	Mollisianitrile	$C_{10}H_8NO_3$	190.0509	190.0504	2.6	8.59	B30
5	Linaloyl glucoside	$C_{16}H_{29}O_6$	317.1970	317.1964	1.9	8.60	B10, B30
6	Scabroside B	$C_{17}H_{29}O_9$	377.1814	377.1812	0.5	8.60	B10
7	Cornolactone B	$C_{10}H_{13}O_4$	197.0813	197.0814	-0.5	8.60	B20
8	Lepidotol E	$C_{29}H_{31}O_6$	475.2139	475.2121	3.8	11.71	R30
9	Melicarpinone	$C_{13}H_{12}NO_3$	230.0811	230.0817	-2.6	11.72	R20, R30
10	9,10-Dihydroxy-2-decanoic acid	$C_{10}H_{17}O_4$	201.1119	201.1127	-4.0	11.72	R10, R30, B20
11	3-Methoxytyramine-BX	$C_{18}H_{21}N_2O_6$	361.1394	361.1400	-1.7	11.73	B20
12	Annonamine	$C_{19}H_{22}NO_2$	296.1664	296.1651	4.4	17.36	R20, B20
13	Magnoflorine	$C_{20}H_{24}NO_4$	342.1713	342.1705	2.3	17.36	R10, R20, R30, B20, B30
14	Licoflavone B	$C_{25}H_{25}O_4$	389.1743	389.1753	-2.6	18.67	R20, R30, B30
15	Gelsemium 3	$C_{20}H_{25}N_2O_6$	389.1731	389.1713	4.6	18.67	R10
16	Unknown	$C_7H_{21}N_{10}O_3$	293.1793	293.1798	-1.7	18.88	R10, R20, R30, B30,
17	Feruloyl putrescine	$C_{14}H_{21}N_2O_3$	265.1540	265.1552	-4.5	21.91	R30, B10, B30
18	Amycocyclopiazonic acid	$C_{18}H_{21}N_2O_2$	297.1596	297.1603	-2.4	22.19	B30
19	Unknown	$C_{13}H_{36}N_5O_6$	358.2670	358.2666	1.1	22.86	R30
20	Hydroxyoctadecadienoic acid	$C_{18}H_{31}O_3$	295.2272	295.2273	-0.3	23.36	B30
21	Dioseptemloside A	$C_{45}H_{75}O_{18}$	903.4980	903.4953	3.0	23.37	B10, B30, R30
22	Colisporifungin	$C_{47}H_{67}N_8O_{10}$	903.4980	903.4980	0.0	23.37	B10
23	6α-Hydroxy-7-oxo-ferruginol	$C_{20}H_{23}O_3$	311.1660	311.1647	4.2	23.98	CO, R10,
24	Unknown	$C_{46}H_{55}O$	623.4249	623.4253	-0.6	23.64	R20
25	14β-Hydroxymeloyunine	$C_{19}H_{23}N_2O_2$	311.1748	311.1760	-3.9	23.98	R10, R30, B10, B30
26	Gagunin B	$C_{38}H_{61}O_{11}$	693.4197	693.4214	-2.5	24.30	R30, B10
27	Aromadendrin glucoside derivative	$C_{37}H_{57}O_{12}$	693.3846	693.3850	-0.6	24.34	CO
28	Manoalide	$C_{25}H_{33}O_4$	397.2382	397.2379	0.8	24.52	R20
29	Methylsarpagine	$C_{20}H_{25}N_2O_2$	325.1927	325.1916	3.4	24.56	CO, R20, B20
30	Polyphyllin V	$C_{39}H_{61}O_{12}$	721.4189	721.4163	3.6	24.81	R20
31	Unknown	$C_{46}H_{57}O_7$	721.4078	721.4104	-3.6	24.82	CO
32	Terpecurcumin Q	$C_{36}H_{43}O_6$	571.3061	571.3060	0.2	26.05	R30
33	Granulatamide B	$C_{24}H_{35}N_2O$	367.2755	367.2749	1.6	27.82	B20
34	Gelliusterol E	$C_{25}H_{35}O_2$	367.2620	367.2637	-4.6	27.82	R30
35	22-Epi-hippuristan-11-one	$C_{28}H_{45}O_5$	461.3263	461.3267	-0.9	29.71	R20, B20
36	Amphidinolide T3	$C_{25}H_{43}O_5$	423.3103	423.3110	-1.7	29.71	R10, R20

pronounced bioactivities and have been used throughout human history as remedies, poisons and psychoactive drugs[21,22]. The alkaloids identified in this study have been established to have health beneficial properties[23,24]. Although, alkaloids are considered to be anti-nutrients because of their action on the nervous system and disrupting or inappropriately augmenting electrochemical transmission[25], the phytochemical screening of *M. myristica* seeds revealed safe levels of alkaloids and other anti-nutrients such as phytates, tannins, saponins, cyanogenic glycosides and oxalates[14,15]. The seed is therefore safe for consumption, hence their use in traditional medicine[8,9]. Moreover, roasting and boiling are effective in eliminating the anti-nutritional factors in foods[26-28].

Only four phytochemicals- Ixoside ($C_{16}H_{19}O_{11}$), 6α-Hydroxy-7-oxo-ferruginol ($C_{20}H_{23}O_3$), Aromadendrin glucoside derivative ($C_{37}H_{57}O_{12}$) and Methylsarpagine ($C_{20}H_{25}N_2O_2$) were identified in the control (raw) sample. However, these compounds have been established not to be deleterious but beneficial to health[29,30]. More phytochemicals were identified in the roasted samples compared to the boiled samples. The reason may be attributed to the fact that boiling led to break down of the plant cell wall which permitted the leakage of cell contents including bio-actives[31] while roasting is a mere gradual evaporation processes[32]. These findings suggest that processing significantly released phytochemicals. The same observation was made by De la Parra *et al.*[33] on the phytochemical constituents of corn for production of Masa, Tortillas and Tortilla Chips. Boiling and roasting induced complex changes in phytochemical composition which is accompanied with change in biological activities. Similar result were reported by Wu *et al.*[34] for Sorghum tea.

Data obtained from the analyses of the methanolic extracts of *M. myristica* seeds are summarised in Table 1. Figure 1-3 shows the chromatogram as affected by processing methods. The next sections present discussions of the chemical characteristics and potential health-benefitting (phytochemical) properties of each of the compounds obtained from the *M. myristica* seed extracts of the current study (arranged according to their aglycone class) and their occurrence in plant species as reported in the literature.

Two iridoid glycosides were found to occur in the *M. myristica* seed extracts of this study. A molecular ion at 387.0934 m/z (molecular formula $C_{16}H_{19}O_{11}$) was identified as ixoside[35]. This compound was identified in C0, R10, R20 and R30 and has been reported in other species from order Lamiales[36,37]. Another signal at m/z 377.1814 was observed at retention time 8.60 (peak 2). The same mass data were reported for Scabroside B by Di *et al.*[38] and were identified only in sample B10. Iridoid Glycosides have laxative and anti-microbial properties. They are also widely believed to possess anti-inflammatory properties, but so far the tests have revealed only a very weak anti-inflammatory effect[39]. Ethanolic extract of *M. myristica* seed possesses antimicrobial activity against *Klebsiella* and *Bacillus* species[40] and possess broad spectrum antibacterial activities against selected Gram-positive and Gram-negative bacteria[41]. It also inhibited *Aspergillus niger* in "Kunun" beverage[42]. This generally confirmed that this seed is highly potent to activities of many microorganisms.

Exact mass measurement of the m/z 455.0956, peak 1 confirmed the compound to be $C_{23}H_{19}O_{10}$, a flavonoid identified in B10. This compound is a rare catechin, (-)-Epicatechin-3-(3''-O-methyl) gallate described in green tea by Miketova *et al.*[43]. The compound with molecular formula $C_{37}H_{57}O_{12}$ was identified as Aromadendrin glucoside derivative[44], another flavonoid which was identified in CO. Flavonoid rich fraction of *M. myristica* seeds exhibited significant *in vitro* anti-inflammatory potentials by stabilizing red blood cell membrane exposed to hypotonic and heat induced lysis with maximum percentage stability of 88% in a biphasic mode of response that is comparable with Ibuprofen a standard anti-inflammatory drug[45]. Antioxidant properties of *M. myristica* have been reported and attributed mainly to its flavonoids composition[46]. Feyisayo and Oluokun[4] evaluated the antioxidant activity of the flavonoid fraction of the seed extract of *M. myristica.* The flavonoid fraction exhibited potent and appreciable anti-oxidant potentials with maximum DPPH-radical scavenging activity (41.20%), hydroxyl radical scavenging activity (46.34%). It also exhibited a significant $p<0.05$ reduction of Fe^{3+} to Fe^{2+} (64.64%). It exhibited a dose dependent protective effect against free radical induced haemolysis of red blood cells with maximum protection and inhibition of lipid peroxidation and free radical generation in liver homogenate.

The ion detected at m/z 378.1700 (peak 2) at 8.59 min, according to Zhang *et al.*[47] corresponds to Oxiamycin. It is a dimeric indolo-sesquiterpene identified in R30. Indolo sesquiterpenes are a group of natural products isolated from plants that exhibit various activities, such as antibacterial, anti-parasitic and anti-human immuno deficiency virus (HIV), as well as inhibitory activities against lipid droplet and non-steroidal progestin biosynthesis[48-50]. The phenolic compounds in *M. myristica* are responsible for the anti-septic, antifungal or bactericide properties of the plant[51]. An antibiotic with the molecular formula $C_{10}H_8NO_3$ (retention time 8.59 min, peak 2) was identified in B30. It is Mollisianitrile [(3-(3,5-Dihydroxy-4-methoxyphenyl) propiolonitrile, which contains a reactive propiolonitrile moiety believed to be

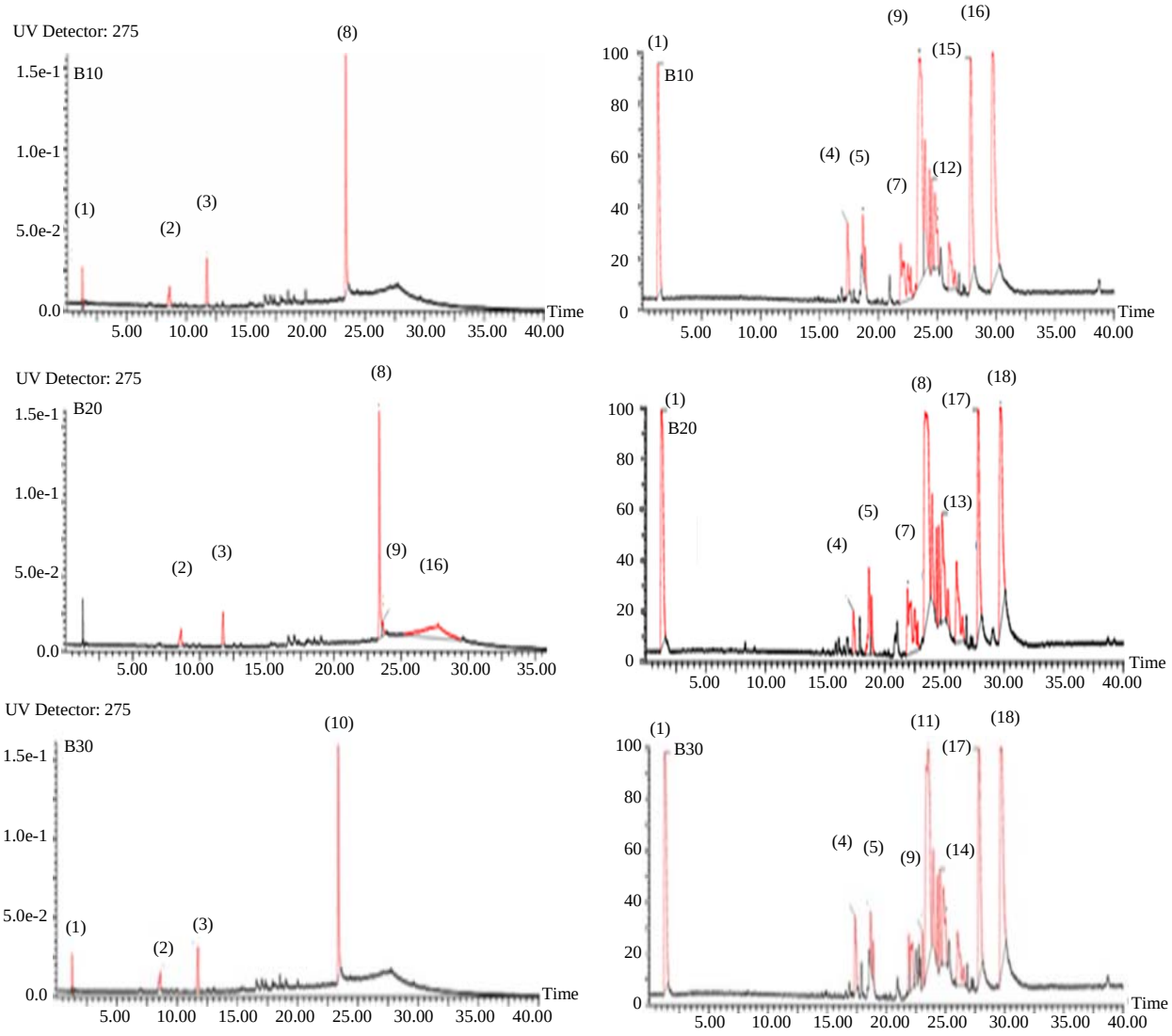

Fig. 1: Chromatogram of boiled *M. myristica* seed extracts

responsible for its antibiotic activities[52]. A signal at m/z 317.1970 was observed at 8.60 min. This compound showed a molecular formula $C_{16}H_{29}O_6$. According to Uchiyama *et al.*[53], it is Linaloyl glucoside, a terpenic glycoside identified in B10 and B30.

Another signal detected at m/z 197.0813 was revealed at retention time 8.60 min and identified in B20. The same mass data was reported for an iridoid aglycone, Cornolactone B, by He *et al.*[54]. Cornolactone B is the first natural cis-fused tricyclic dilactone iridoid containing both a five- and a six-membered lactone ring[54]. Iridoid aglycones have been reported to possess antimicrobial and antitumor properties[55]. Ukaegbu-Obi *et al.*[56] reported that the seed extracts of *M. myristica* possess some antimicrobial activities which can be employed in the development of novel therapeutic agents against the test organisms. They assessed the antimicrobial activity of *M. myristica* seeds on four selected human pathogens, *Escherichia coli* (*E. coli*), *Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa* (*P. aeruginosa*) using Disc diffusion technique for *in vitro* antibacterial screening. They observed that the most susceptible bacterium were *E. coli*, while the most resistant bacterium was *P. aeruginosa* and the minimum inhibitory concentration result showed that the seed extracts of *M. myristica* was bacteriostatic. At a retention time of 11.71 min, a signal with m/z 475.2139 was detected. This confirmed the presence of a phenylcoumarin derivative named Lepidotol E[57] which was identified in R30.

Nine alkaloids were identified in the processed and raw *M. myristica* methanolic extracts. Two of them, Melicarpinone ($C_{13}H_{12}NO_3$)[25] and Annonamine ($C_{19}H_{22}NO_2$)[31] were identified in R20 and R30. Compound with molecular formula $C_{20}H_{24}NO_4$

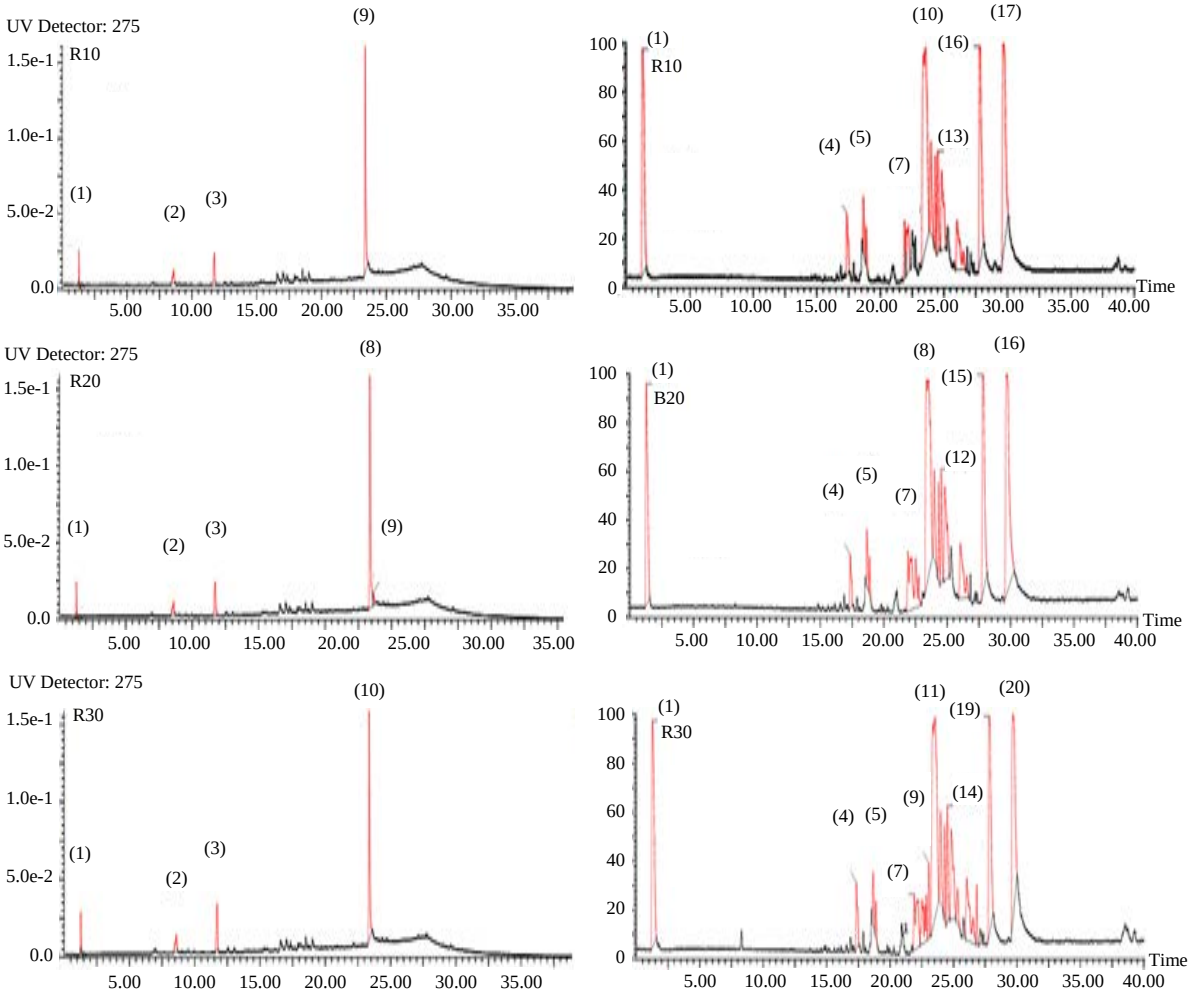

Fig. 2: Chromatogram of roasted *M. myristica* seed extracts

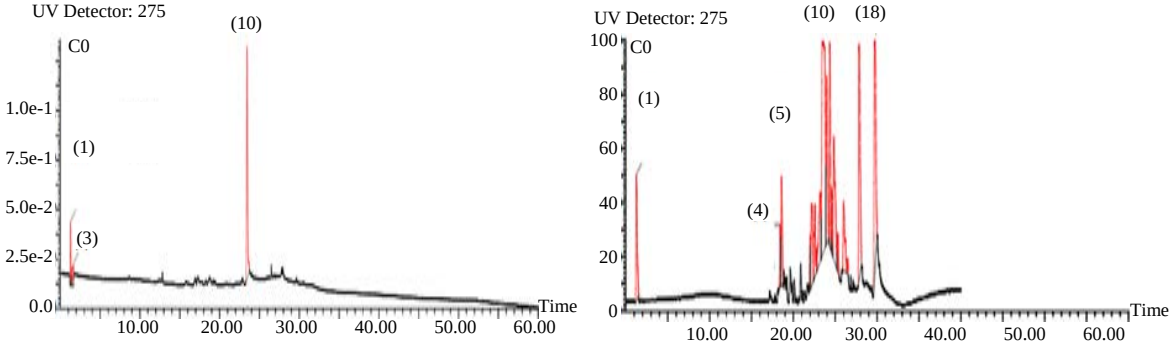

Fig. 3: Chromatogram of raw *M. myristica* seed extracts

and m/z 342.1713, magnoflorine[58] was identified in R10, R20, R30, B20 and B30. The compound with m/z 389.1731 (retention time, 18.67, peak 5), Gelsemium 3, a new gelsedine-type oxindole alkaloid, previously isolated from the stems and leaves of cultivated Carolina jasmine (*Gelsemium sempervirens* A_{IT}. f.)[24] was identified in R10. A compound identified in R30, B10 and B30 presented signals at m/z 265.1540, molecular formula, $C_{14}H_{21}N_2O_3$. The mass data was compared with the data obtained by Kim *et al.*[59] and it was identified as Feruloyl putrescine. Amycocyclopiazonic acid (molecular formula $C_{18}H_{21}N_2O_2$, m/z 297.1596) was identified in B30. It is a new bacterial indole alkaloid related

to the cyclopiazonic acid class, which has previously only been found in fungi[29]. The metabolite 14β-Hydroxymeloyunine (m/z 311.1748, molecular formula $C_{19}H_{23}N_2O_2$), a monoterpenoid indole alkaloid previously isolated from leaves and twigs of *Melodinus yunnanensis* was identified in R10, R30, B10 and B30[60]. According to Kumar *et al.*[61] compound with the molecular formula $C_{20}H_{25}N_2O_2$ (m/z 325.1927, retention time 24.56min, peak 13) is Methylsarpagine identified in CO, R20 and B20. Compound with a molecular ion 367.2755 and molecular formula $C_{24}H_{35}N_2O$ was identified as Granulatamide B, a new tryptamine derivative, marine indole alkaloid[62,63]. This compound was detected in B20. It had previously been isolated from the 2-propanol extract of the soft coral *Eunicella granulate* and showed moderate *in vitro* cytotoxicity against a panel of 16 human tumour cell lines[62]. Other reports on the phytochemical screening of the seeds of this plant also revealed the presence of phenolic compounds, including flavonoids and tannins, cyanogenic glycosides, alkaloids, arocine, lactose, terpene, resins, fiberro-latic oils, anthraquinones, saponins, steroids, oxalates and phytates[14,15]. The mechanism of inhibitory action of these alkaloids and phenolic compounds on micro-organisms may be due to impairment of variety of enzyme systems, including those involved in energy production, interference with the integrity of the cell membranes and structural component synthesis[64,65].

The ES-MS signals at m/z 201.1 (identified in R10, R30, B20) and m/z 295.2 (identified in B30) were 9,10-Dihydroxy-2-decanoic acid[66] and Hydroxy octadecadienoic acid[67] respectively. Hydroxy fatty acids are drawing much attention recently due to their anti-inflammatory, antimicrobial and cytotoxic activities[68,69]. GC-MS analytical report for the chloroform fraction of *M. myristica* seed showed the presence of n-hexadecanoic acid, 9-Octadecanamide, cis-9-Hexadecenal, acetic acid, cis-vaccenic acid, campesterol and butyl-9-Octadecenoate amongst others[70].

The compound 3-Methoxytyramine-BX, at m/z 361.1394, a new betaxanthin (yellow chrome alkaloids) detected at a retention time of 11.73 min (peak 3), was identified in B20 and the same mass spectra was reported for this compound by Schliemann *et al.*[71]. Betaxanthins display potent antioxidant, anti-inflammatory and chemo-preventive activity *in vitro* and *in vivo*[72]. Ishola *et al.*[73] carried out a study to investigate the antinociceptive and anti-inflammatory effects of the hydroethanolic seeds extract of *Monodora myristca* (HMM) in male albino rats. They reported that HMM possesses antinociceptive effect mediated through interaction with opioidergic, serotonergic and dopaminergic systems and an anti-inflammatory action through inhibition of inflammatory mediator's release. Their study established the scientific basis for its use in the management of pain and inflammatory conditions in traditional medicine. The flavone, Licoflavone B[74] was identified in R20, R30 and B30 respectively. A Cholestane glycoside, Dioseptemloside A, (molecular formula $C_{45}H_{75}O_{18}$, m/z 903.4980)[75], was identified in B10, B30 and R30. An antifungal lipopeptide was also detected in this study. According to Ortiz-Lopez *et al.*[76] peak 8 with m/z $C_{47}H_{67}N_8O_{10}$ (retention time, 23.37 min) corresponds to Colisporifungin an antifungal lipopeptide which was identified in B10. *M. myristica* extracts inhibited the growth of mycelium, the formation of conidial spores and chlamydospores of *Sclerotium rolfsii*, thereby reducing the number of propagation units of this fungus in the medium[65,77]. Also, Enabulele *et al.*[5] reported that the aqueous and ethanolic extracts of *M. myristica* seeds, were active against both Gram-negative and Gram-positive organisms- *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and *Salmonella typhi.* Its methanol and dichloromethane extract was active against mites and traditionally used against scabies[78]. The metabolite, 6α-Hydroxy-7-oxo-ferruginol[79] a new phenolic diterpene with the molecular formula $C_{20}H_{23}O_3$ was identified in CO and R10. Gagunin B, ($C_{38}H_{61}O_{11}$, 693.4197)[80] a poly-oxygenated diterpene was identified in R30 and B10. It had been reported to have anti-tumour properties[81].

Manoalide ($C_{25}H_{33}O_4$) with m/z 397.2382 (retention time, 24.52 min, peak, 12), a sesterterpene[82] and Polyphyllin V, a saponin[83] were identified in R20. Manoalide is an analgesic, possesses potent anti-inflammatory activity, irreversibly inhibits human synovial fluid PLA2[84] as well as bee[85] and cobra venom PLA2 and inhibits ornithine decarboxylase[86]. These activities have led to the use of manoalide in the prevention of post-surgical adhesion of tissues and as a molecular tool in the study of psoriasis and skin cancers[82]. *M. myristica* seeds are a natural source of anti-inflammatory agent[73]. Flavonoid rich fraction of *M. myristica* seeds inhibited heat induced albumin denaturation with maximum inhibition of 75.38% in a concentration dependent manner that is comparable with aspirin and showed an anti-lipoxygenase activity range from 19-71% which is comparable to that produced by indomethacin[45].

Terpecurcumin Q ($C_{36}H_{43}O_6$) with m/z 571.3061 (retention time, 26.05, peak 17) and Gelliusterol E ($C_{25}H_{35}O_2$, m/z 367.2620, retention time, 27.82, peak 19) were identified in R30. Terpecurcumin Q a novel terpene-conjugated curcuminoid exhibited IC_{50} of 3.9 μM against MCF-7 human breast cancer cells[87] while Gelliusterol E inhibited the formation and growth of chlamydial inclusions in a dose-dependent manner with an IC_{50} value of 2.3 μM[88]. Essential oils obtained by hydrodistillation of fruits of

M. myristica exerted cytotoxic activity against cancer and normal cell lines with more pronounced effect on neoplastic cells in the majority of cases[89].

The metabolite 22-Epi-Hippuristan-11-one ($C_{28}H_{45}O_5$, m/z 461.3263), a new highly oxygenated spiroketal steroid hippuristanol, was identified in R20 and B20[90]. Hippuristanols have been reported to have significant cytotoxicity against several cancer cell lines[91]. Other reports on the phytochemical screening of the seeds of this plant also revealed the presence of steroids[14,15]. Amphidinolide T3, a new 19 membered macrolide, which was reported to exhibit cytotoxicity against murine lymphoma L1210 (IC_{50}:7) and human epidermal carcinoma KB cells (IC_{50}: 10)[92] was identified in R10 and R20. It presented signals at m/z 423.3103 with molecular formula, $C_{25}H_{43}O_5$. The biological properties, unique structural features and scarcity of supply of Amphidinolides have already attracted immense synthetic interest[93].

Unidentified compounds: Molecular ions at m/z 293.1798 (observed exact mass 293.1793), m/z 358.2666 (observed exact mass 358.2670), m/z 623.4253 (observed exact mass 623.4249) and m/z 721.4104 (observed exact mass 721.4078) eluting at 18.88, 22.86, 23.64 and 24.82 min, respectively could not be identified.

CONCLUSION

The combination of the accurate mass spectroscopy (MS) in the determination of elemental composition and the ability of Ultra High Performance Liquid Chromatography (UPLC) to separate isomeric compounds provided a powerful tool for the identification of 32 metabolites present in raw and processed *Monodora myristica* seed flour. The study revealed that boiling and roasting affected the release of metabolites in the flour. The metabolites identified have been reported to possess phytochemical properties. Furthermore, different unknown substances were reported and other analyses (such as NMR) will be necessary to identify these compounds and to establish if they are phytochemicals. *M. myristica* seed can be considered a good source of phytochemicals. The phytochemical potential of this plant should be investigated further as the current data indicate that its seeds have abundant and diverse phytochemicals, as well as the fact that the plant is significant in oriental traditional medicine.

SIGNIFICANCE STATEMENT

This study reveals that boiling and roasting can have beneficial effects on the phytochemical constituents of *M. myristica*. It has also shown that thermal processing induced the release of phytochemicals especially roasting process as the raw extracts had the least number of phytochemicals. The results of this study will help other researchers in the development of new functional foods from whole *M. myristica* seeds or its extracts.

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