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Comparative analysis of phenolic profile of *Monodora myristica* and *Monodora tenuifolia*

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This study sought to identify phenolics present in the seeds of *Monodora myristica* and *Monodora tenuifolia* by Gas chromatography (GC) coupled to Flame ionization detector (FID). GC-FID analysis identified fifty-three different types of phenolics in both *M. myristica* and *M. tenuifolia* seeds. Predominant phenolics are Myristicin (RT: 13.78), Caffeic acid (RT: 14.16), Safrole (RT: 11.37), Methyl Eugenol (RT: 12.32), Catechin (RT: 3.63), Elemicin (RT: 13.69), Quercetin (RT: 25.05), Kaempherol (RT: 21.52), Methyl Isoeugenol (RT: 12.69) and Eugenol (RT: 11.70). It was observed that *M. myristica* is rich in Myristicin (42.60%), Caffeic acid (23.39%), Elemicin (3.82%) and Eugenol (1.02%), while *M. tenuifolia* is rich in Safrole (11.86%), Methyl Eugenol (6.28%), Catechin (5.27%), Quercetin (2.97%), Kaempherol (2.27%) and Methyl Isoeugenol (1.45%). However, the two species of *Monodora* contained virtually the same kinds of phenolics but in varying quantities with *M. myristica* having a higher total phenolic content (1478.32 mg/100 g) than *M. tenuifolia* (1171.52 mg/100 g). Both species are promising sources of phenolics.

Key words: *Monodora myristica*, *Monodora tenuifolia,* phenolics.

INTRODUCTION

Phenolics are secondary metabolites that are synthesized by plants during development, and in response to biotic and abiotic stresses such as infections, wounding, ultraviolet (UV) radiation (Lattanzio et al., 2006; Putrussa et al., 2013). They are present in all plants and contribute to the development of color, taste and palatability, as well as the defense system of plants (Tarnai et al., 1994). They share a common structural feature: an aromatic ring bearing at least one hydroxyl substituent (Croteau et al., 2000). Historically, some of these phenolics were considered as antinutrients (Fattouch et al., 2007) but recent reports on the antioxidant and antimicrobial properties of phenolics lead to a rethinking among food scientists (Mercy et al., 2009).

Phenolics have an array of health promoting benefits; they are of current interest due to their important biological and pharmacological properties, especially the antiinflamatory, antioxidants, antimutagenic and anticarcinogenic activities (Ehsan et al., 2012). It is therefore important to analyze the composition of phenolic compounds in foods before their health-promoting properties can be adequately studied. The analysis of phenolic compounds in plant samples is difficult because of the great variety of their structure and the lack of appropriate standards (Magalhães et al., 2009; Huang et al., 2007). Although extracts of spices

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Figure 1. Photographs of *Monodora myristica* (a) and *Monodora tenuifolia* (b).

have been shown to be rich in phenolics (Wojdjlo et al., 2007), literature is scarce on the nature of phenolics present in these spices, therefore, this study sort to report the different types of phenolics present in the plants *Monodora myristica* and *Monodora tenuifolia*.

M. myristica (Figure 1a) and *M. tenuifolia* (Figure 1b) belong to the custard apple family of flowering plants Annonaecea. Their generic name was derived from the Greek word meaning “single gift”. *M. myristica* is widely distributed from Africa to Asia, Central and South America and Australia (Omobuwajo et al., 2003). It is native to West, Central and East Africa extending from Sierra leone to Uganda, Kenya, Kongo and Angola (Keay, 1989). It grows well in the evergreen forest of West Africa and most prevalent in the Sothern part of Nigeria (Adegoke and Akinsanya, 1970). Its local name include: Ehuru or Ehiri (Igbo), Ariwo (Yoruba), Jamaica nutmeg, African nutmeg, Calabash nutmeg, and Airama. *M. myristica* is used in Ivory Coast to treat hemorrhoids, stomach ache and febrile pains. *M. myristica* seeds are aromatic and are used after grinding to powder as condiments in food providing a flavour resembling that of nutmeg (Ekeanyanwu et al., 2010). The seeds are also used as an aromatic and stimulating addition to medicines and to snuff (Burkill, 1985; Ekeanyawu et al., 2010). When ground to powder, the kernel is used to prepare pepper soup as stimulant to relieve constipation and control passive uterine hemorrhage in women immediately after child birth (Okafor, 1987; Udeala, 2000; Iwu, 2002).

M. tenuifolia is found in the forest region of East Indies, West Indies, Malaysia, Sirlanka and Africa (Talaji, 1965). In Africa, *M. tenuifolia* is widely distributed along the West Coast (Adeoye et al., 1986) and occurs in Nigeria, Guinea, Cameroun, Gabon and Zaire (Congo Democratic Republic) where it is used as an ornamental plants in food and as medicines (Burkhill, 1985). In traditional medicine, it is widely used to relieve toothache, dysentery, (Nelson, 1979; Adeoye et al., 1986), dermatitis, headache and as cermifuge (Adeoye 1986). *M. tenuifolia* seeds (Figure 1b) are aromatic and are used as ingredient in herbal medicine in Southern Nigeria and as spices and flavour (Ogutimein et al., 1989). When roasted, the ground seed of *M. tenuifolia* are rubbed on the skin for skin diseases (Irvine, 1961).

MATERIALS AND METHODS

Collection of plant materials

Seeds of *M. myristica* were purchased from a local market in Ile-Ife, while the seeds of *M. tenuifolia* were collected from the Zoological garden, Obafemi Awolowo University. These seeds were identified and authenticated at Ife herbarium Obafemi Awolowo University, Ile-Ife.

Preparation of plant material

The seeds were air-dried in the laboratory for a period of 7 days after which they were decocted to free the kernel which were later ground to powder with an electronic blending machine.

Extraction of phenolics

About 50.0 mg of the sample was extracted with 5 ml of 1M NaOH

for 16 h on a shaker at ambient temperatures as described by Kelley et al. (1994). After extraction, the sample was centrifuged (5000 × g), rinsed with water, centrifuged again, and the supernatants were combined and placed in a disposable glass test tube and heated at 90°C for 2 h to release the conjugated phenolic compounds as supported by Whitehead et al. (1983). The heated extract was cooled, titrated with 4M HCL to pH < 2.0, diluted to 10 ml, with deionised water, and centrifuged to remove the precipitate. The supernatant was kept for subsequent purification and the residue was further extracted with 5 ml of 4M NaOH, heated to 160°C in Teflon. After cooling, the mixture was filtered. Supernatant was collected and the residue was washed with water (deionised). The supernatants were combined and adjusted to pH < 2.0 with 4M HCL. The filtrates were combined for further purification.

Purification of extracted phenolics

An aliquot (5 to 15 ml) of various supernatants was passed through a conditioned Varian (Varian Assoc., Harbor City, CA) Bond Elut PPL (3 ml, size with 200 mg packing) solid phase extraction tube at ~1 ml min^{-1} attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were then placed under a vacuum (-60 KPa) until the resin was thoroughly dried after which the Pas were eluted with 1 ml of ethyl acetate into Gas chromatography (GC) auto sampler vials. The PPL tubes were conditioned by first passing 2 ml of ethyl acetate followed by 2 ml water (Ph < 2.0).

Derivatisation

The concentrated extract of about 2 ml in the GC vials was derivatized by adding BSTFA (bis (trimethylsily) trifluoroacetamide). The silicone septum corked vial was lowered into the water bath with hanger to stand upright in the water bath with a magnetic stirrer at 45°C for the derivitization period of 10 min.

Chromatographic conditions

A GC-Flame ionization detector (FID) (GC-FID) HP 6890 series equipped with HP INNOWax capillary column (30 m × 0.25 mm × 0.25 µm) was used. The injector in split mode (20:1) was set at 250°C, injection volume was 1 µl and the detector temperature was 320°C. Nitrogen was used as the carrier gas. The oven was initially set at 50°C, raised to 210°C at 8°C/min and maintained for 4 min. The temperature was again increased to 260°C at a rate of 12°C/min and held for 4 min.

RESULTS AND DISCUSSION

The total phenolic content of seeds of *M. myristica* was estimated to be 1478.32 mg/100 g, while the phenolic content of the seed of *M. tenuifolia* was estimated to be 1171.52 mg/100 g. The total phenolic content of *M. tenuifolia* was lower than that of *M. myristica*. The levels of total phenolics in both plant seeds were higher than the levels of total phenolics in some vegetables and spices reported by previous works of Lee et al. (2003), Abdou et al. (2010), and George and Osioma (2011). Variations in phenolic content as reported in the literature could partially be associated with the method of extraction employed. It has been noted that extraction yield of phenolics using ethanol was 2 to 3-fold lower than that with methanol (Abdou et al., 2010). It might also be due to differences in the analytical techniques employed as well as maturity of the plant (Howard et al., 2000).

About fifty-three different types of phenolics were identified in the seeds of *M. myristica* (Table 1) and *M. tenuifolia* (Table 2). Both seeds contained virtually the same kinds of phenolics but in varying amounts. Table 3 shows the ten prominent phenolics (Myristicin, Caffeic acid, Safrole, Methyl Eugenol, Catechin, Elemicin, Quercetin, Kaempherol, Methyl Isoeugenol and Eugenol) identified in the seeds of *M. myristica* and *M. tenuifolia* and their percentage composition, while Figure 2 shows the structures of prominent phenolics identified in seeds of *M. myristica* and *M. tenuifolia*. It was observed that *M. myristica* is richer in Myristicin, Caffeic acid, Elemicin and Eugenol compared to *M. tenuifolia* that is richer in Safrole, MethylEugenol, Catechin, Quercetin, Kaempherol and Methyl Isoeugenol. Plants have been known to vary within and among species in the types and concentrations of phenolics due to variables in plant growth, soil, weather condition and the age of the plant (Scalbert et al., 2005). It is known that amongst other factors such as maturity stage or light exposure, phenolic composition also varies with the cultivar and their different structures or levels are likely to have different functional properties (Huang et al., 2007; Magalhaes et al., 2009).

Phenolics, which are present in foods, have attracted a great deal of attention recently due to reports of the role they play in preventing diseases (Shahidi and Naczk, 2004; Fattouch et al., 2007; Sara et al., 2010). They are important due to their ability to serve as antioxidants which are widely found in secondary products of medicinal plants (Wang et al., 2008; Ehsan et al., 2012). The antioxidant activity of phenolics is attributed to their redox properties which play a role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994; Rice-Evans et al., 1997; Aranya et al., 2013). To the best of our knowledge information is scarce on the nature of phenolics present in the spices *M. myristica* and *M. tenuifolia*. However, several researchers (Abdou et al., 2010; George and Osioma, 2011; Lovet and Enebi, 2012) have reported the presence of phenolics in *M. myristica* and *M. tenuifolia* seeds.

Conclusion

The results obtained in this present work suggest that *M. myristica* and *M. tenuifolia* are good sources of phenolics, indicating that inclusion of these spices in human diet could contribute to potential health benefits.

Conflict of Interests

The authors have not declared any conflict of interests.

Table 1. Phenolic compounds identified in the seed of *Monodora myristica* by GC-FID showing their retention time (RT), area pA*s, amount (mg/100 g) and names.

pK	Retention time (min)	Area (pA*s)	Amount (mg/100 g)	Name
1	3.63	207.97	72.21	Catechin
2.	6.78	18.53	1.48e-4	Phenol
3.	7.35	9.52	7.59e-5	Phenylacetic acid
4.	7.65	21.18	1.69e-4	Salicylic acid
5.	7.96	8.61	6.86	Myrcene
6	8.78	4.00	3.19e-5	Cinnamic acid
7	9.69	48.59	13.21	Protocatechuic acid
8.	10.01	1.91	1.28e-2	Carvacrol
9.	10.11	2.97	2.36e-5	Gentisic acid
10.	10.79	1.64	1.98e-2	p-coumaric acid
11.	11.17	3.38	4.00e-2	Vanillic acid
12.	11.37	101.88	165.39	Safrole
13.	11.70	3.75	15.08	Eugenol
14	11.96	4.11	3.27e-5	Isoeugenol
15.	12.32	4.28	79.76	Methyly Eugenol
16.	12.69	3.44	20.49	Methyl Isoeugenol
17.	13.21	10.02	7.98e-5	Gallic acid
18.	13.69	23.50	56.50	Elemicin
19.	13.78	5.24	629.72	Myristicin
20.	14.16	42.60	345.79	Caffeic acid
21	14.94	15.48	5.60e-2	Ferulic acid
22.	15.39	35.18	2.80e-4	Syringic acid
23.	15.50	4.87	7.87e-3	Piperic acid
24.	16.14	16.35	1.17e-2	Sinapinic acid
25.	16.55	24.36	9.82e-3	Daidzein
26.	17.52	4.06	1.10e-3	Coumestrol
27	18.39	3.33	3.25e-3	Genistein
28.	18.77	5.36	1.10e-2	Apigenin
29.	19.00	6.35	5.06e-5	Naringenin Chalcone
30.	19.31	3.77	3.77e-5	Naringenin
31.	19.67	9.39	2.88e-2	Shogaol
32.	20.61	2.34	1.92e-3	Glycitein
33.	21.52	3.40	32.95	Kaempferol
34.	21.83	1.03	1.11e-3	Luteolin
35.	22.39	1.92	2.98e-3	Capsaicin
36.	22.70	2.06	1.64e-5	Epicatechin
37.	23.22	3.37	2.92e-5	Epigallocatechin
38.	23.34	9.30e-1	9.01e-4	Gingerol
39.	24.14	9.74e-1	9.44e-3	Myricetin
40.	24.50	8.44e-1	2.50e-3	Isorhamnetin
41.	25.05	49.42	40.11	Quercetin
42.	25.26	3.63	2.96e-4	3-o-caffeoylquinic
43.	25.53	3.90	6.25e-3	Chlorogenic acid
44.	26.24	4.46	7.01e-3	Rosmarinic acid
45.	26.93	3.49	2.83e-3	Curcumin
46.	27.20	1.72	2.76e-3	Miquelianin
47.	27.48	9.09e-1	1.44e-3	Eriocitrin
48.	28.25	2.48	4.03e-3	Rutin
49.	29.00	5.27	4.29e-6	Papain
50	29.31	4.08	3.25e-5	Phenyl-6-o-malonyl-beta-D- glucoside

Table 1. Contd.

51.	29.48	9.21	7.33e-5	4-o-methyl-epi-gallocatechin
52.	30.06	48.07	3.83e-4	Epi-gallocatechin-3-O-gallate
53	30.26	19.03	1.52e-4	Lupeol

Table 2. Phenolic compounds identified in the seed of *Monodora myristica* by GC-FID showing their retention time (RT), area (pA*s), amount (mg/100g) and names.

pK	Retention time (min)	Area (pA*s)	Amount (mg/100 g)	Name
1.	3.63	232.21	61.76	Catechin
2.	6.94	74.18	5.91e-4	Phenol
3.	7.37	26.50	2.11e-4	Phenylacetic Acid
4.	7.65	34.60	2.75e-4	Salicylic Acid
5.	7.95	12.71	5.06	Myrcene
6.	8.77	23.44	1.87e-4	Cinnamic Acid
7.	9.69	11.25	8.79	Protocatechuric Acid
8.	10.13	17.92	1.43e-4	Gentisic Acid
9.	10.28	14.00	9.39e-2	Carvacrol
10	10.79	11.47	1.39e-1	p-coumaric Acid
11	11.14	17.79	2.11e-1	Vanillic Acid
12	11.37	68.89	138.80	Safrole
13	11.70	18.79	11.13	Eugenol
14	12.02	15.21	1.21e-4	Isoeugenol
15	12.25	8.67	73.54	Methyl Eugenol
16	12.69	5.69	16.93	Methyl Isoeugenol
17	13.19	$9.47e^{-1}$	7.54e-6	Gallic Acid
18	13.61	5.06	39.51	Elemicin
19	13.83	31.00	490.55	Myristicin
20	14.16	32.44	263.30	Caffeic Acid
21	14.97	61.00	2.21e-2	Ferulic Acid
22	15.25	16.61	1.32e-4	Syringic Acid
23	15.54	23.92	3.86e-3	Piperic Acid
24	16.25	17.59	1.26e-2	Sinapinic Acid
25	16.55	42.96	1.73e-3	Daidzein
26	17.45	18.27	4.96e-3	Coumestrol
27	18.40	31.58	3.08e-3	Genistein
28	18.78	21.81	4.46e-2	Apigenin
29	19.03	10.38	8.26e-5	Naringenin Chalcone
30	19.35	10.59	1.06e-5	Naringenin
31	19.67	23.70	7.23e-2	Shogaol
32	20.51	25.74	2.12e-3	Glycitein
33	21.53	13.82	26.62	Kaempferol
34	21.85	10.94	1.19e-3	Luteolin
35	22.32	7.47	1.16e-3	Capsaicin
36	22.86	5.22	4.16e-5	Epicatechin
37	23.08	2.25	1.80e-5	Epigallocatechin
38	23.41	3.44	3.34e-4	Gingerol
39	24.03	1.55	1.50e-3	Myricetin
40	24.57	4.43e-1	1.31e-3	Isorhamnetin
41	25.05	2.20	34.82	Quercetin
42	25.26	1.74	1.42e-4	3-o-caffeoylquinic

Table 2. Contd.

43	25.54	1.95	3.12e-3	Chlorogenic Acid
44	26.38	1.84	2.89e-3	Rosmarinio Acid
45	26.94	6.57	5.32e-3	Curcumin
46	27.08	8.12	1.30e-3	Miquelianin
47	27.48	18.83	2.93e-3	Eriocitrin
48	28.32	35.66	1.62e-4	Rutin
49	29.00	9.01	7.96e-7	Papain
50	29.30	25.94	8.00e-7	4-o-methyl-epi-gallocatechin
51	29.69	17.38	7.96e-7	Phenyl-6-o-malonyl-beta-D-glucoside
52	29.84	18.67	7.96e-6	Lupeol
53.	30.00	36.71	7.96e-6	Epigallocatechin-3-o-gallate

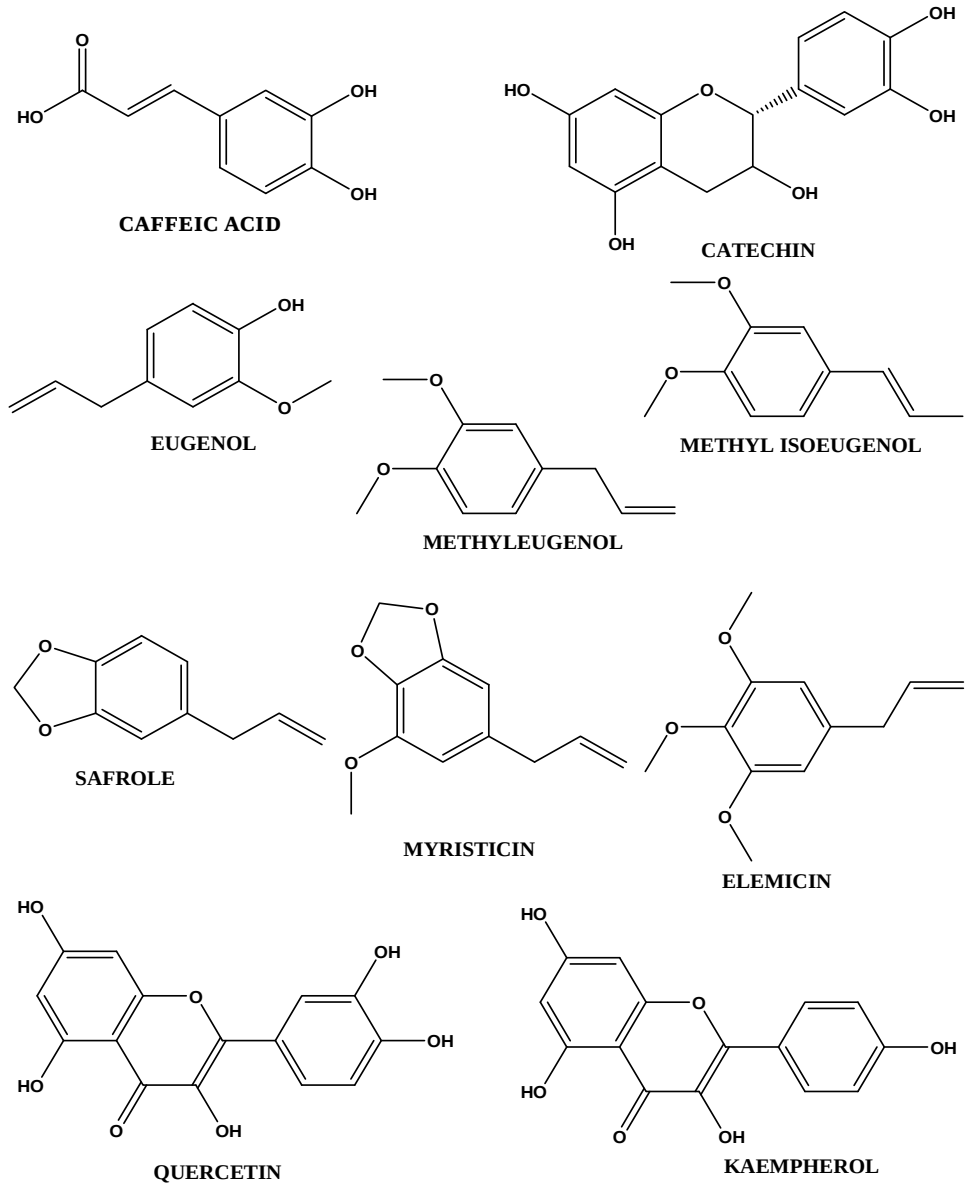

Figure 2. Structures of major phenolics identified in the seeds of *Monodora myristica* and *Monodora tenuifolia*

Table 3. Major phenolics in the seeds of *Monodora myristica* and *Monodora tenuifolia* as identified by GC-FID and their percentage composition.

S/N	Identified phenolics	% composition	
		M. myristica	*M. tenuifolia*
1.	Myristicin	42.60	41.87
2.	Caffeic acid	23.39	22.48
3.	Safrole	11.19	11.86
4.	Methyl Eugenol	5.40	6.28
5.	Catechin	4.88	5.27
6.	Elemicin	3.82	3.37
7.	Quercetin	2.71	2.97
8.	Kaempherol	2.23	2.27
9.	Methyl Isoeugenol	1.39	1.45
10.	Eugenol	1.02	0.95

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