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Original Research Article

GC-MS Fingerprinting of Methanolic Extract of *Moringa oleifera* Stem, Leaf and Root

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ABSTRACT

Moringa oleifera is a tropical tree species found in both the tropics and sub-tropics. It is famous for its medicinal and nutritional qualities. The leaf is mostly used in different modes. The research aims at comparing the compounds present in the extract of the stem, leaf and root, as well as the residue and unextracted samples using GC-MS. The leaf, stem and root of the tree were collected, dried, ground and extracted with 70% methanol. The extracts, residue and unextracted samples were analysed using GC-MS. Library matches of 80% quality and above were considered. Fifteen (15) compounds were identified in both the leaf and the stem extracts each while seventeen (17) compounds were identified in the root extract with ten (10) of the compounds common to the three samples at different concentrations. Similarly, nine (9) compounds each were found to be common to the extract, residue and unextracted sample for all the three plant parts analysed. Antimicrobial compounds like cyclotrisiloxane, hexamethyl and cyclotetrasiloxane, octamethyl were identified in all the samples and eicosane identified in both the leaf and stem extract. Phytol and neophytadiene with antioxidant anti-inflammatory activities were found in the leaf extract only. The solvent tried to concentrate some compounds in the extract especially the stem sample. However, many compounds were not successfully extracted especially from root. These results will give more insight to the naturopath or alternative medicine users who decide between using different solvents or whole herb. Also, unidentified peaks because of no quality/ any library matches need more work for identification.

Keywords: *Moringa oleifera*, Gas Chromatography-Mass Spectrometry (GC-MS), naturopath, alternative medicine, methanolic extract, residue.

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Introduction

Moringa oleifera is a tree popularly called "drumstick tree" or "miracle tree." The name "miracle tree" is because of its nutritional component and use for traditional medicines. The different parts of the tree are being utilized as food, fencing, fodder and medicine which includes conditions like high blood pressure, labour pains, diabetes, arthritis, fever, diabetes, epilepsy, skin infection and wound.[1–5] Ethnobotanical studies have shown its inclusion in different traditional medicines .[6]The leaf has been found to be rich in vitamins, minerals and protein (including essential proteins).[7–9] Its *in-vivo* and *in-vitro* antioxidant properties have also been shown.[2] These components and antioxidant activities contributes to its medicinal and nutritional uses.
The stem of *Moringa oleifera* is not left out. Although not as often used like the leaf but it also has notable attributes and applications. The stem is fibrous and sturdy and is used in building and as source of fibre. It is used for mats baskets and ropes in some places because of its fibrous nature. It is also used in water purification. When crushed, it is a natural anticoagulant and thus remove impurities and turbidity of water. It was shown to be effective in removing microbes, dyes and even heavy metal. It has also been shown to possess antifungal and anti-bacterial activity.[10,11]

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Furthermore, *Moringa oleifera* bark (stem) is also used in medicine because of its anti-inflammatory and antimicrobial properties. The root has also been shown to possess antibacterial and antifungal activity.[10,11] Both the root and bark are reported to be used in dental carries/toothache, common cold and pains.[12] However, the stem and root are not as widely used as the leaves.
As more uses of the part of the tree is being discovered, the research aims at comparing the different component present in the methanol extract of the leaf, stem and root and in what quantity using GC-MS (Gas Chromatography-Mass Spectrometry). Also, it aims to assess the effectiveness of the solvent used for extraction by comparing the extracts, residues and unextracted samples (whole samples that have not been extracted) of the three plant parts. This is the first for *Moringa oleifera* to the best of our knowledge. These would shed more light on more interchangeable usage of the different plant parts and the difference in the compounds available if whole sample or extract is used. Therefore, the detection capacity of a mass spectrophotometer is combined with the separating ability of a gas chromatography to identify the bioactive compounds. GC-MS is usually used for identification of active compounds in medicinal plants.[13–15] This is because of the special role GC-MS plays in analyzing phytochemicals and their chemotaxonomic studies of those with biologically active components.[16,17]

Materials and Methods

The leaves, stem (bark) and root of authenticated *Moringa oleifera* tree were collected in September 2022 and deposited in the herbarium with voucher number UHAE 2022012. The leaves were air dried while the stem and root were sun dried. They were then ground and a part of each were extracted with 70% methanol of analytical grade. The extract and residue were then dried. The dried extract, dried residue and unextracted sample (ground but not extracted) were analysed using a GCMS. Hexane and acetone (twice the amount of sample) were added to the sample. This was ultrasonicated at 27^0C for 20 minutes. They were then filtered and concentrated for the GC-MS analysis.

Phytochemical levels were determined by operating MSD scan mode. An Agilent 7820A gas chromatograph which is coupled to 5975C inert mass spectrometer that has triple axis detector with electron-impact source (Agilent Technologies) was used. 5% Phenyl Methyl Siloxane was used to coat HP-5 capillary column for the stationary phase. The carrier gas Helium was at an initial pressure of 1.4902 psi, a constant flow (1.4871 mL/min) and average velocity of 44.22 cm/sec. The injection mode was splitless at temperature of 300^0C for 1µL of samples. The mass spectrometer was used in electron-impact ionization mode at 70 eV, with the ion source set to 230 ℃, the quadrupole at 150 °C, and the transfer line at 280 ℃. The different peaks detected were matched with National Institute of Standards and Technology's library (NIST14.LIB). The library matches with not less than 80% quality or the first three in the case of more than three matches with more than 80% quality were considered.[18–20]

Results and Discussion

The GC-MS chromatogram of the leaf extract is shown in Figure 1A. Prominent peaks and their respective retention times are shown on the chromatogram. Library matches of the chromatogram peaks with quality of not less than 80 % or first 3 matches were considered (Table 1). Also, the prominent peaks from the chromatogram (not shown) of both the residue and the unextracted with quality match are shown (only those also common to the extract) (Table 1). Fifteen peaks were identified with Cyclotrisiloxane, hexamethyl- having the greatest area of 14.76 % and Bicyclo[3.1.1]heptane, 2,6,6-trimethyl- having the least area of 1.17 %. While nine compounds were found to be common to the extract, residue and unextracted. Percentage peaks of five out of the nine were found to be highest in the extract although they were just slightly different. The percentage peak of the residue was the least in many except in nonadecane which had 0.217 % peak area as against the unextracted and the extract which had 0.109 and 0.172 respectively. However, some prominent peaks in the leaf extract chromatogram do not have a match from the library (Figure 1A). For example, peaks with retention time of 17.226 and 23.360.

Figure 1B and Table 2 showed the chromatogram of the GC-MS of the stem extract and the library matches of not less than 80 % or first 3 matches respectively. Cyclotrisiloxane, hexamethyl like the leaf extract had the greatest peak area with 19.86 % while two retention times (7.346 min and 17.707 min) had the least peak of 0.8% peak area. These were identified to be mesitylene or Benzene, 1,2,3-trimethyl- (7.346 min) and octadecane, 2-methyl- or triacontane or heneicosane Cyclononasiloxane (17.707 min). Also, the prominent peaks from the chromatogram (not shown) of the unextracted with quality match are shown on the Table (only those also common to the extract) (Table 2). None of the matched compounds were found to be common between the extract, the residue and the unextracted. However, eight (8) compounds are found common to both the extract and the unextracted samples and the peak areas in the extract are more than that of the unextracted sample in all the nine compounds.

The GC-MS chromatogram of the root extract is presented in Figure 1C. The prominent peaks with 80% quality match or the first three matches where more than 3 matches have a quality of not less than 80 % are shown (Table 3). Seventeen peaks had matches from the library with Cyclotrisiloxane, hexamethyl- also having the highest peak area of 19.45 % and the least was 1.28 % which was identified to be cyclotetradecane or n-nonadecanol-1 or bromoacetic acid, hexadecyl ester. Peak with retention time of 25.106 did not have a quality match. Nine peaks were found to be common to the extract, residue and the unextracted. The peak area of the unextracted was highest in four of the peaks while the extract was highest in the remaining five although with not so much difference when compared to the unextracted.

Some of these compounds with quality match have been found to be useful in medicine. The medicinal activities of the plant could be attributed to these compounds while some of them indicate possible toxicity. This therefore calls for caution in the usage of the plant. For example, toluene, one of the compounds identified in the three samples is a neurotoxic compound that have been shown to generate reactive oxygen species in the brain. Exposure to it can cause irritation to the eye, nose, headache, muscle fatigue, insomnia etc. if exposed to a sufficiently high dose.[21,22] The leaves had 5.6 %, the stem 6.94 % and the root 6.41% of this compound (Tables 1, 2 and 3). This compound was absent only in the stem residue.

However, cyclotrisiloxane, hexamethyl, cyclohexasiloxane dodecamethyl, and cyclotetrasiloxane, octamethyl which have been identified in a potent antifungal fraction of Streptomyces strain against *Rhizoctonia solani* in tobacco leaf [23] were identified in the extract of the three samples as well as their residues and unextracted compounds except the residue of the stem. These compounds also possess anti-bacterial and anti-oxidant activities.[24] Further, Benzene, 1,2,3-trimethyl- found in all the samples have also been identified in an endophytic fungus extract shown to exhibit a wide antimicrobial activity spectrum.[13]

Phytol, identified only in the leaf extract with 5.38 % peak area has been reported to have anti-inflammatory, diuretic, antioxidant, and anticancer activity.[25,26] Also, neophytadiene with reported antipyretic, anti-inflammatory, analgesic, antimicrobial and antioxidant activity was identified in the leaf (absent in the residue). Neophytadiene compound have also been found in plants with antipyretic, vermifugic and analgesic activity.[27,28] It had also been reported to possess neuropharmaceutical effect (anticonvulsant and anxiolytic like activities).[29,30] The compound is also a known antimicrobial and anti-inflammatory agent which is said to inhibit the growth of most common fungi and many gram-positive bacteria. It has been described to have good anti-inflammatory, antipyretic, analgesic, antioxidant, and antimicrobial properties. [27,28]

Eicosane, identified in only the leaf and stem as well as the residue of leaves (Tables 1 and 2) are known antifungals.[23] It has also been found to possess antioxidant, antimicrobial antiglycemic cytotoxic and insecticidal activities.[14,31] In addition, stigmast-4-en-3-one a reported compound with hypoglyemic effect[32] was identified only in the stem extract.

Furthermore, hexadecanoic acid, methyl ester identified only in the root extract have been said to play a role in controlling epilepsy.[33] It has also been shown to have antioxidant, 5- alpha-reductase inhibiting, anti-fibrinolytic, hemolytic, antimicrobial activity, hypocholesterolemic nematicidal, pesticidal, antiandrogenic flavor, and hemolytic properties.[34,35] It is also a potent competitive inhibitor of phospholipase A2, and as a result, it is thought to be useful in inflammation management. Also, 9-octadecanoic acid identified in the root extract is being said to possess antifungal potential.[36,37] Additionally, dodecanoic acid, 1,2,3-propanetriyl ester which is also only found in the root extract is one of the major component of antidiabetic fraction of *Cnidoscolusa conitifolius*.[38] It has been shown to possess hepatoprotective ability and γ -sitosterol, an epimer of β-sitosterol which has been shown to possess antihyperglycemic effect by increasing insulin production.[39]

The presence of these compounds supports the medicinal properties of the plant parts. However, some of the peaks do not have quality match with the library which shows further work is needed to identify these compounds. Some of these compounds not identified could be novel and also could be responsible for the various medicinal activities found in the different parts of the plant. It could also be a guide to revolution in the use of plant in medicines. Comparison of the identified peaks in the extract, residue and unextracted samples indicates that the solvent, 70% methanol was effective in extracting compounds from the stem sample better than the leaves and the root samples.

Conclusion

The leaf stem and root of *Moringa oleifera* actually contains different biochemicals that have made them relevant in traditional medicines. The research has shown similarities and uniqueness of the different parts of the plant. Some compounds are found common to the different parts of the tree i.e. leaf, root and stem, while some compounds are peculiar to specific parts.

The extraction made some compounds more prominent however, the solvent used seemed not to have notable effect on some other compounds as the solvent used, failed to extract some important compounds effectively while some were well extracted showing concentration of the compounds in the extract. Thus, it is therefore important that residue after extraction should be verified for other

important biochemicals that could be of interest. Furthermore, extraction could be based on biochemical of interest as extraction could be channeled to specific needs.

Although a lot have been carried out on *Moringa oleifera*, it is important that the compounds yet unfolded is explored for better understanding and utilization of the different parts of the tree. The peaks unmatched could be isolated for proper recognition or other methods could be employed to identify them.

Furthermore, the whole plant might be used as herbs except in specific needs as one solvent might not be suitable to extract all the biochemicals of interest especially by the alternative medicine users or the naturopaths. Further studies could also show more of the synergistic effect of some of these compounds which supports the effectiveness of the plant when used as a whole.

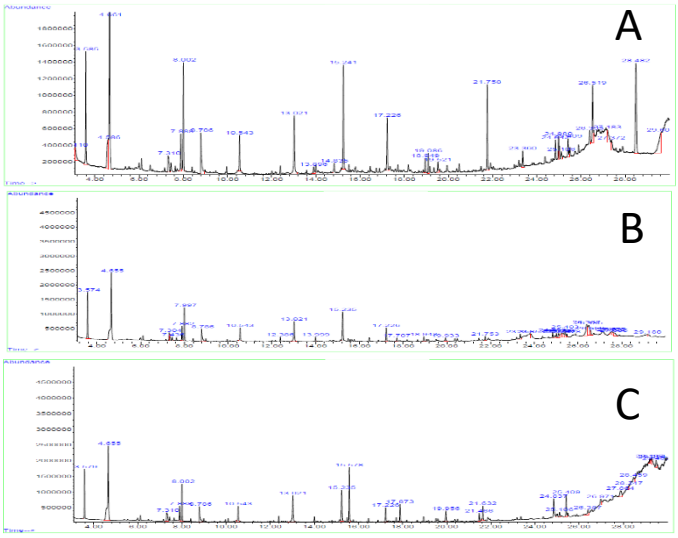

Figure 1: (A) The GC-MS chromatogram of the leaf extract of *Moringa oleifera*
(B) The GC-MS chromatogram of the stem extract of *Moringa oleifera*
(C) The GC-MS chromatogram of the root extract of *Moringa oleifera*

Table 1: Library matches of the identified peaks from the chromatogram of *Moringa oleifera* Leaf extract, residue and unextracted sample with their retention times and percentage peak area.

S/No	RT (min)	Hit Name	Peak Area (%)		
			Extract	Residue	Unextracted
1	3.585	Toluene	5.60	3.25	6.14
		Toluene			
		Toluene			
2	4.586	Cyclotrisiloxane, hexamethyl-	3.51		
.3	4.661	Cyclotrisiloxane, hexamethyl-	14.76	13.05	19.41
4	7.31	Benzene, 1-ethyl-3-methyl-	1.97	1.16	2.40
5	7.888	Mesitylene	2.14		
		Benzene, 1,2,3-trimethyl-			
		Benzene, 1,2,3-trimethyl-			
6	8.002	Cyclotetrasiloxane, octamethyl-	6.90	3.96	6.84
7	8.786	2-Pyrrolidinone, 1-methyl-	3.81		
		2-Pyrrolidinone, 1-methyl-			
8	10.543	Cyclopentasiloxane, decamethyl-	2.29		
		Cyclopentasiloxane, decamethyl-			
9	13.021	Cyclohexasiloxane, dodecamethyl-	5.74	3.19	5.23
		Cyclohexasiloxane, dodecamethyl-			
10	15.241	Cycloheptasiloxane, tetradecamethyl-	7.78	4.13	6.14
		Cycloheptasiloxane, tetradecamethyl-			
11	19.086	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	1.17		
		Neophytadiene			
12	21.758	Phytol	5.38		
13	24.98	Eicosane	1.74		
		Eicosane			

		Heptadecane			
14	26.519	Eicosane	4.70		
		Eicosane			
		Tridecane, 6-propyl-			
15	28.482	Eicosane	9.93	5.74	9.55
	26.513	Nonadecane	0.17	0.22	0.11
		2-Methylpentacosane			

Table 2: Library matches of the identified peaks from the chromatogram of *Moringa oleifera* stem extract, residue and unextracted sample with their retention times and percentage peak area.

			Peak Area (%)		
S/No	**RT (min)**	**Hit Name**	**Extract**	**Residue**	**Unextracted**
1	3.574	Toluene	6.94	2.62	-
		Toluene			
		Toluene			
2	4.655	Cyclotrisiloxane, hexamethyl-	19.86	6.59	-
3	7.304	Benzene, 1-ethyl-2-methyl-	1.37		
		Benzene, 1-ethyl-3-methyl-			
		Benzene, 1-ethyl-3-methyl-			
4	7.436	Mesitylene	0.80		
		Benzene, 1,2,3-trimethyl-			
		Mesitylene			
5	7.882	Benzene, 1,2,3-trimethyl-	2.28	0.89	-
		Mesitylene			
		Mesitylene			
6	7.997	Cyclotetrasiloxane, octamethyl-	6.47	2.72	-
7	8.786	2-Pyrrolidinone, 1-methyl-	3.20	2.81	-
8	10.543	Cyclopentasiloxane, decamethyl-	2.64	1.01	-
		Cyclopentasiloxane, decamethyl-			
9	13.021	Cyclohexasiloxane, dodecamethyl-	5.71	2.36	-
10	13.999	Tetradecane	0.82		
		Tetradecane			
11	15.235	Cycloheptasiloxane, tetradecamethyl-	6.91	2.80	-
12	17.707	Octadecane, 2-methyl-	0.80		
		Triacontane			
		Heneicosane			
13	18.943	Cyclononasiloxane, octadecamethyl-	1.11		
14	19.933	Eicosane	0.96		
		Octadecane, 3-ethyl-5-(2-ethylbutyl)-			
15	26.485	Stigmast-4-en-3-one	4.98		

Table 3: Library matches of the identified peaks from the chromatogram of *Moringa oleifera* root extract residue and unextracted sample with their retention times and percentage peak area.

			Peak area (%)		
S/No	**RT (min)**	**Hit Name**	**Extract**	**Residue**	**Unextracted**
1	3.579	Toluene	6.41	7.63	8.36
		Toluene			
		Toluene			
2	4.655	Cyclotrisiloxane, hexamethyl-	19.45	25.68	24.07

3	7.31	Benzene, 1-ethyl-3-methyl- Benzene, 1-ethyl-2-methyl- Benzene, 1-ethyl-2-methyl-	2.31	1.42	1.58
4	7.888	Mesitylene Mesitylene Benzene, 1,2,3-trimethyl-	2.22	2.50	2.62
5	8.002	Cyclotetrasiloxane, octamethyl-	6.29	8.31	7.66
6	8.786	2-Pyrrolidinone, 1-methyl- 2-Pyrrolidinone, 1-methyl- 2-Pyrrolidinone, 1-methyl-	3.38	2.13	2.81
7	10.543	Cyclopentasiloxane, decamethyl- Cyclopentasiloxane, decamethyl-	2.95	3.06	3.25
8	13.021	Cyclohexasiloxane, dodecamethyl-	6.37	6.63	6.76
9	15.235	Cycloheptasiloxane, tetradecamethyl-	6.62	8.30	7.34
10	15.578	Dodecanoic acid, methyl ester Dodecanoic acid, methyl ester Pentadecanoic acid, methyl ester	8.08		
11	17.873	Methyl tetradecanoate Methyl tetradecanoate Tridecanoic acid, 12-methyl-, methyl ester	2.66		
12	19.956	Hexadecanoic acid, methyl ester Hexadecanoic acid, methyl ester Pentadecanoic acid, 14-methyl-, methyl ester	2.24		
13	21.466	Cyclotetradecane n-Nonadecanol-1 Bromoacetic acid, hexadecyl ester	1.28		
14	21.632	9-Octadecenoic acid (Z)-, methyl ester 9-Octadecenoic acid (Z)-, methyl ester 9-Octadecenoic acid, methyl ester, (E)-	2.35		
15	24.837	2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,12,13,14-octahydro-6-oxabenzocyclododecen-5-one	4.79		
16	25.409	Bis(2-ethylhexyl) phthalate Bis(2-ethylhexyl) phthalate Phthalic acid, di(oct-3-yl) ester	3.93		
17	28.459	Dodecanoic acid, 1,2,3-propanetriyl ester	1.84		

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