Chemical composition from the leaf extracts of *Momordica angustisepala* with its antibacterial, antifungal and antioxidant activities

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ABSTRACT

The chemical constituents of weighed, air-dried leaf samples of Momordica angustisepala were examined. The bioactivity of hexane, ethyl acetate and methanol extracts of the two plants were tested against ten (10) strains of bacteria and fungi, and their antioxidant activities were studied. The GC-MS characterisation of n-hexane extract of Momordica angustisepala leaves revealed twenty-one (21) chemical compounds with n-tetracontane being the most abundant constituent with 13.86%. Ethyl acetate extract of the plant showed a total of nineteen (19) compounds with n-hexadecanoic acid (18.63 %) being the most abundant constituent of the extract. The characterisation of the methanol extract of Momordica angustisepala leaves gave thirty-two (32) compounds with clionasterol, eremophila-1(10), 11-diene and α-amyrinas principal constituent with 19.45 % each in the extract.

KEY WORDS: Antioxidant activity, hexadecanoic acid, GC-MS.

INTRODUCTION

Momordica angustisepala (Cucurbitaceae) commonly occurs in closed, deciduous and semi-deciduous forest. It is also habited at the edge of secondary forest or old plantations, disturbed areas and by the roadsides[1]. The plant is a dioecious climber, tendrils unequally branched, its stem is more or less puberulous, bifid, spirally coiled with a straight part. Its leaves are simple, alternates, with moderately long petiole, puberulous or almost glabrous with blade broadly ovate or ovate-oblong, membranous, scabrid-puberulous on both surfaces[2,3]. *Momordica angustisepala* is a natural fibre and it is reported to be used in the reinforcement of polymers. Ikechukwu in 2014 used the fibre of the plant (*Momordica angustisepala)* to strengthen polypropylene by forming a fibre-polymer composite using compression moulding. In another research by Anthony *et al.*, 2015[4], the influence of *Momordica angustisepala* fiber on the tensile strength of concrete produced from conventional concrete constituents and partially replaced by recycled concrete aggregate is reported to be significant. The root extract of *Momordica angustisepala* has been shown to possess abortifacient

properties in female albino mice, hence toxic to pregnant women[1,3].

This research focuses on the antimicrobial and antioxidant properties of these plant leaf extracts, as there are very few research reports on the antimicrobial and antioxidant properties of the plant's leaves to the best of our knowledge.

MATERIALS AND METHODS

The aerial parts of the *Momordica angustisepala* were sourced from Ondo and Oyo states, Nigeria. They were identified by the plant taxonomist, Mr. Bolu Ajayi of the Department of Plant Biology, University of Ilorin where voucher specimens (UIH/007/1239) were deposited in the herbarium. The leaf parts of *M. angustisepala* were air-dried and crushed into smaller pieces using mortar and pestle. The plant samples were weighed and extracted using serial exhaustive extraction method by moving from a non-polar (n-hexane) solvent to a medium polar solvent (ethyl acetate) and then to a polar solvent (methanol).

Phytochemical screening

Preliminary phytochemical examination of the crude extracts was carried out using the modified methods described by Pranshant, *et al.,* 2011[5].

Antimicrobial assays

Test Microorganisms: Cultures of six human pathogenic bacteria made up of four Gram-negative and two Gram-positive were used for the antibacterial assay. The Gram-negative bacteria are *Salmonella typhii, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonae*, while *Bacillus subtilis* and *Staphylococcus aureus* are the gram-positive bacteria used. The four fungi utilized for the antifungal assay are; *Candida albicans, Aspergillus niger, Rhizopus stolon* and *Penicillium notatum.* All the microorganisms used are clinical strains from the Department of Medical Microbiology (University College Hospital, Ibadan) and were screened in the Laboratory of Pharmaceutical Microbiology department, University of Ibadan, Ibadan, Nigeria.

Media

Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. N-hexane, ethyl acetate and methanol were also used in solubilizing the extracts and act as negative controls in the assays.

Antimicrobial agents used

Gentamicin (10 µg/*mL*) and tioconazole (0.7 mg/*mL*) were employed as standard reference drugs in these studies.

Determination of Antimicrobial activity

Agar diffusion-Ditch method (for bacteria)

Overnight, culture of each organism was prepared by taking two wire-loop of the organism from the stock, and each inoculated into 5*mL* of sterile nutrient broth and incubated for 24 hrs at 37^{o}C. Then 0.1 *mL* of each organism was taken from the overnight culture and put into a 9.9 *mL* of sterile distilled water to obtain 10^{-2} M inoculum concentration of the test

organism. 0.2 *mL* was taken from the diluted test organism (10^{-2}) into the prepared sterile nutrient agar cooled to about 45^{o}C, then poured into sterile petri dishes and allowed to solidify for about 60 min. A sterile cork borer of 8 mm diameter was used to make 8 wells on the media according to the number of the diluted extracts for the experiment. The graded concentrations (6.25 – 200 mg/*mL*) of the extracts were put into each well and separated from the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2 hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 24 hr at 37^{o}C.

Agar diffusion-Surface method (fungi)

A sterile sabouraud dextrose agar was prepared accordingly and skeptically poured into the sterile plates in triplicates and was properly solidified. 0.2 *mL* of the 10^{-2} inoculum concentration of the test organism was spread on the surface of the agar while using a sterile petri dish to cover all the surface of the agar. Eight wells were bored by using a sterile cork-borer of 8 mm diameter. The graded concentrations of the extracts were separately put into each well with the controls. All the plates were left on the bench for 2 hrs to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated at 25^{o}C for 72 hrs[6,7].

Antioxidant Activity

Since DPPH is widely used to test the ability of compounds to trap free radical or hydrogen donors, and to evaluate antioxidant activity, the ability of the plant samples to scavenge DPPH free radicals was assessed by the standard method adopted with suitable modifications[8]**.** The stock solutions of extracts were prepared in methanol to achieve the concentration of 1 mg/*mL*. Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.99 µg/*mL* The absorbance was measured in triplicate at varying concentrations and the mean absorbance was determined. Parallel to examination of the antioxidant activity of plant extracts, the value for the standard compound (Ascorbic acid) was obtained (Table 2) and compared to the values of the antioxidant activity, the percentage inhibitions of the serial concentrations of the methanol DPPH extracts and that of the standard which was determined at different concentrations using the expression below:

$$\%\ inhibition = \left(\frac{A\ of control - A\ of sample}{A\ of control}\right) \times 100$$

The IC_{50} values (Inhibition concentration at 50%) were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

GC-MS analysis of the extracts

GC-MS analysis of *M. angustisepala* extracts was performed with Agilent 19091GC plus automatic sampler system coupled with a quadruple Mass Spectrometer 433HP-5MS. Compounds were separated in HP5MS column fused with phenylmethylsilox, (length; 30 m x 250 µm; film thickness 0.25 µm). Samples were injected at a temperature of about 250 ^{o}C with a

split ratio of 10:1, the flow rate of helium being 1*mL*/min[9,10].

RESULTS AND DISCUSSION

Phytochemical Screening

The preliminary phytochemical screening of the crude extracts of *M. angustisepala* revealed the presence of bioactive compounds such as phenolics, tannins, flavonoids, fats and oils, terpenoids, alkaloids, steroids, glycosides and carbohydrate as shown in Table 1. The presence of these bioactive compounds is an indication that this plant may possess some pharmacological activities.

Table 1 : Phytochemical Screening of hexane, ethyl acetate and methanol extracts of the leaves of *M. angustisepala.*

Chemical constituents	Hexane extract	Ethyl acetate extract	Methanol extract
Alkaloids	-	-	-
Glycoside	-	+	+
Carbohydarate	--	-	-
Flavonoids	-	+	+
Tannins	-	-	+
Saponins	-	-	+
Terpenoids	+	+	+
Steroids	+	+	+
Anthraquinone	-	-	-
Fat & Oils	+	+	-
Phenols	-	-	-
Protein	-	-	-

KEYS: + = Present; - = Absent

Antimicrobial activity

The n-hexane and methanol extract of *M. angustisepala* are active against all test organisms (bacteria and fungi) except *Penicillium notatum* and *Rhizopus stolonifer*, at moderate to high concentrations (50-200 mg/mL) with mean zone of inhibition above 10 mm compared with the positive controls (Tables 2). On the other hand, the ethyl acetate extract of *M. angustisepala was* active, without exception against all test organisms (bacteria and fungi) at moderate to high concentrations (25-200 mg/mL) with mean zone of inhibition above 10 mm compared with the positive controls (Table 2).

These inhibitory properties give credence to the fact that leaf parts *M. angustisepala* exhibit antibacterial and antifungal activities and hence can be used for the treatment of various illnesses caused by the strains of bacteria and fungi used in this research.

Table 2: Antimicrobial activity of n-hexane, ethyl acetate and methanol extracts of *M. angustisepala* leaves

Microorganism	Mean zone of Inhibition (mm)																							
	n-hexane extract								Ethyl acetate extract								Methanol extract							
S. aureus	17	15	13	10	-	-	-	37	19	17	14	12	10	-	-	38	19	15	13	10	-	-	-	36
E. coli	17	14	12	10	-	-	-	39	19	17	14	12	10	-	-	38	18	14	12	10	-	-	-	36
B. subtilis	17	15	13	10	-	-	-	37	19	16	14	12	10	-	-	37	17	14	12	10	-	-	-	38
P. aeruginosa	15	12	10	-	-	-	-	37	19	16	14	12	10	-	-	37	17	14	11	-	-	-	-	36
K. pneumonae	15	13	11	-	-	-	-	37	17	14	12	10	-	-	-	38	14	12	10	-	-	-	-	38
S. typhi	17	14	12	10	-	-	-	38	19	16	42	10	-	-	-	39	16	14	10	-	-	-	-	40
C. ablicans	17	14	10	-	-	-	-	27	17	14	12	10	-	-	-	28	15	13	10	-	-	-	-	26
A. Niger	15	12	10	-	-	-	-	26	18	15	12	10	-	-	-	27	15	12	10	-	-	-	-	28
P. notatum	-	-	-	-	-	-	-	28	17	14	12	10	-	-	-	28	-	-	-	-	-	-	-	28
R. stolonifera	-	-	-	-	-	-	-	27	15	12	10	-	-	-	-	28	-	-	-	-	-	-	-	28
Conc of extracts (mg/mL)	200	100	50	25	12.5	6.25	-ve	+ve	200	100	50	25	12.5	6.25	-ve	+ve	200	100	50	25	12.5	6.25	-ve	+ve

Key: +ve = Gentamicin 10 µg/*mL* (bacteria), Tioconazole 70% (fungi); -ve = Solvent of dilution

Antioxidant activity

The ability of the plant's extracts (n-hexane, ethyl acetate and methanol) to scavenge DPPH radicals and reducing their effects was analyzed. The results of this analysis are as shown in the tables and figures below:

Table 3: DPPH Antioxidant activity and % inhibition of n-hexane extract of *M. angustisepala* leaves. 0.432 is the absorbance of control

Conc. (µg/*mL*)	Absorbance	Absorbance	Absorbance	AV±SD	%I inhibition
1000	0.078	0.077	0.078	0.078±0.000	67.50
500	0.038	0.042	0.042	0.041±0.001	82.98
250	0.029	0.029	0.032	0.030±0.001	87.44
125	0.045	0.049	0.059	0.051±0.005	78.66
62.6	0.065	0.061	0.060	0.062±0.002	74.06
31.25	0.085	0.081	0.077	0.081±0.003	66.10
15.62	0.085	0.084	0.083	0.084±0.000	64.85
7.8	0.088	0.086	0.087	0.087±0.000	63.59
3.9	0.081	0.081	0.080	0.081±0.000	66.24
1.95	0.093	0.092	0.093	0.093±0.000	61.22

Table 4: DPPH Antioxidant activity and %inhibition of ethyl acetate extract of *M. angustisepala* leaves. 0.432 is the absorbance of control

Conc (μg/*mL*)	Absorbance	Absorbance	Absorbance	AV±SD	%I inhibition
1000	0.418	0.420	0.419	0.419±0.000	43.49
500	0.203	0.203	0.204	0.203±0.000	30.36
250	0.105	0.106	0.108	0.106±0.001	63.58
125	0.078	0.080	0.079	0.079±0.000	72.95
62.6	0.077	0.080	0.083	0.080±0.002	72.60
31.25	0.098	0.103	0.107	0.103±0.003	64.35
15.62	0.107	0.107	0.107	0.107±0.000	63.35
7.8	0.109	0.111	0.111	0.110±0.000	62.21
3.9	0.117	0.116	0.117	0.117±0.000	60.05
1.95	0.108	0.109	0.105	0.107±0.001	63.24

Table 5: DPPH Antioxidant activity and %inhibition of methanol extract of *M. angustisepala* leaves. 0.432 is the absorbance of control

Conc (μg/*mL*)	Absorbance	Absorbance	Absorbance	AV±SD	%I inhibition
1000	0.223	0.225	0.223	0.224±0.0012	17.16
500	0.158	0.156	0.158	0.157±0.0012	41.73
250	0.071	0.074	0.074	0.073±0.0017	72.96
125	0.05	0.049	0.049	0.049±0.0006	81.73
62.5	0.057	0.056	0.056	0.056±0.0006	79.14
31.25	0.076	0.087	0.087	0.083±0.0064	69.14
15.62	0.093	0.094	0.093	0.093±0.0006	65.43
7.8	0.105	0.11	0.111	0.109±0.0032	59.75
3.9	0.103	0.102	0.1	0.102±0.0015	62.35
1.95	0.103	0.111	0.11	0.108±0.0044	60.00

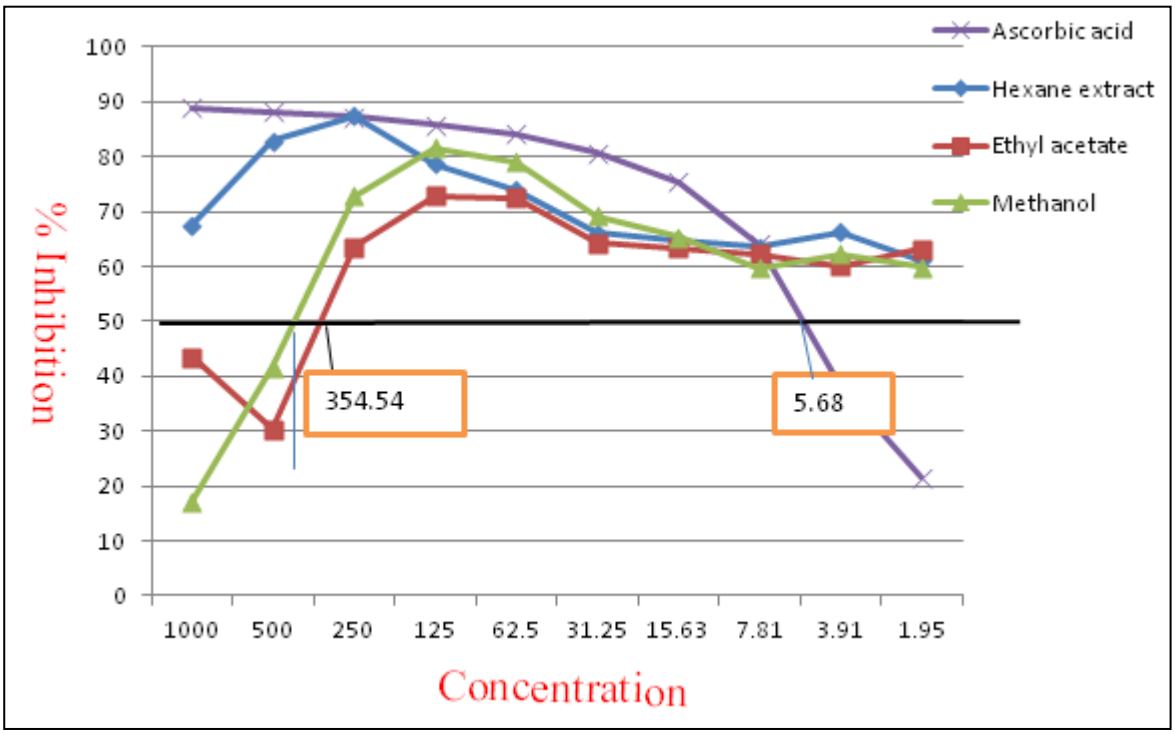

Figure 1: Antioxidant activities of leaf extracts of *M. angustisepala*

Tables 3-5 and figure 1 showed that the various extracts *of M. angustisepala* leaves exhibits significant antioxidant activities by scavenging DPPH radicals in a dose-dependent manner with n-hexane extract exhibiting highest percentage inhibition (87.44%) at 250 µg/mL. At 125 µg/mL, both ethyl acetate and methanol extracts displayed highest percentage inhibition at 72.95% and 81.73% respectively. These results lend support to the folkloric use of leaves of *M. angustisepala* in treating various diseases like cancer, diabetes e.t.c. that involve damaging of cells by free radicals

GC-MS Results

Hexadecanoic acid (8.68%), 9-octadecenamide (10.88%), methyl-24-methyl heptaecosanoate (6.72%) and tetratetracontane (13.86%) were the principal constituents from hexane extract of *M. Augustisepala* leaves while ethyl acetate extract of the plant also showed the presence of hexadecanoic acid (18.63%), gamma-sitosterol (16.76%) and beta-amyrin (8.51%)as the major components. Meanwhile the principal compounds of methanol extract of the plant were clionasterol, eremophila-1(10),11-diene and α-amyrin constituting 19.45%, 19.45% and 14.28% respectively. The presence of these major compounds with other trace of components in the extracts might be responsible for its antimicrobial and free radical scavenging properties (Table 2 – 5).

Table 6: GC-MS Analysis of n-hexane leaf extract of *M. angustisepala*

S/N	Compound name	Peak Area %	Molecular Weight (g/mol)	Retention time (min)
1	Menthol	4.65	156	5.742
2	n-Dodecane	1.74	170	6.109
3	3,7-dimethyldecane	1.18	170	7.218
4	n-Tetradecane	6.11	198	8.775
5	n-Hexadecane	2.19	226	10.008
6	n-Heptadecane	5.63	240	11.475
7	2-ethyl-2-methyl-1-tridecanol	1.58	242	13.430
8	n-Octadecane	2.71	254	14.712
9	6,10,14-trimethyl-2-pentadecanone	1.19	268	15.268
10	2-ethyl-2-methyl-1-tridecanol	1.49	242	16.319
11	n-Hexadecanoic acid	8.68	256	16.748
12	n-Heneicosane	1.32	296	17.142
13	Oleanitrile	2.09	263	17.917
14	7-Hexadecenal	6.99	238	18.591
15	Octadecanamide	2.65	283	18.907
16	9-Octadecenamide	10.88	281	20.536
17	Bisoflex 81	4.82	390	22.059
18	1-methylbutyl docosanoate	5.19	410	23.872
19	2-methyl tetracosane	5.27	352	24.720
20	Methyl-24-methyl heptacosanoate	6.72	424	25.485
21	n-Tetratetracontane	13.86	618	26.656

Table 7: GC-MS Analysis of ethylacetate leaf extract of *M. angustisepala*

S/N	Compound Name	Peak Area %	Molecular Weight (g/mol)	Retention time
1	6,10,14-trimethyl-2-pentadecanone	1.53	268	15.258
2	n-Hexadecanoic acid	18.63	256	16.771
3	Ethyl palmitate	2.93	284	17.038
4	Phytol	2.36	296	18.300
5	Dicloroacetic acid,tridec-2-ynyl ester	5.19	306	18.575
6	Octadecanoicacid,2-(2hydroxyethoxy)ethyl ester	4.70	284	18.803
7	Phytol, acetate	1.63	338	19.305
8	Octadecamethylcyclononasiloxane	1.27	666	20.004
9	9-octadecenamide	2.30	281	20.525
10	Octadecamethylcyclononasiloxane	1.47	666	21.234
11	Bisoflex 81	2.54	390	22.058
12	Octadecamethylcyclononasiloxane	2.44	666	22.374
13	Gamma-sitosterol	16.76	414	22.718
14	Octadecamethylcyclononasiloxane	4.73	666	23.434
15	Alpha-amyrin	8.51	426	24.272
16	Octadecamethylcyclononasiloxane	4.79	666	24.450
17	2-methyl octacosane	3.12	408	24.724
18	Octadecamethylcyclononasiloxane	6.87	666	25.640
19	n-Tetratetracontane	4.06	618	26.658

Table 8: GC-MS Analysis of methanol leaf extract of *M. angustisepala*

S/N	Compound Name	Peak Area %	Molecular Weight (g/mol)	Retention time
1	Tridecane	0.10	184	8.708
2	Caryophyllene	0.55	204	9.091
3	Alpha-Muurolene	0.17	204	10.067
4	Cadina-3,9-diene	0.38	204	10.353
5	1,4-Methano-1H-cyclohepta[d]pyridazine, 4,4a,5,6,7,8,9,9a-octahydro-10,10-dimethyl	0.35	192	11.249
6	Sulfurous acid, 2-ethylhexyl hexyl ester	0.48	278	11.450
7	Methylundecane	0.16	170	13.203
8	Methylundecane	0.13	170	13.409
9	3,7-dimethylundecane	0.29	184	14.693
10	Hexahydrofarnesyl acetone	0.94	268	15.245
11	Palmitic acid, methyl ester	1.90	270	16.249
12	n-hexadecanoic acid	1.24	256	16.691
13	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	1.39	374	18.138
14	Phytol	2.06	296	18.288
15	Methyl stearate	0.51	298	18.407
16	(Z)-14-Tricosenyl formate	0.83	366	18.540
17	18-Methyl-nonadecane-1,2-dio, trimethylsilyl ether	0.60	458	18.630
18	3-Dodecanol	0.44	186	18.877
19	Cyclononasiloxane, octadecamethyl	0.62	666	19.993
20	Adogen 73	4.16	281	20.510
21	Cyclononasiloxane, octadecamethyl	1.01	666	21.222
22	3-Bromo-2-propynyl palmitate	0.96	372	21.729
23	9-t-Butyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	7.51	224	22.042
24	Cyclononasiloxane, octadecamethyl	2.58	666	22.360
25	Clionasterol	19.45	414	22.699
26	Eremophila-1(10), 11-diene	19.45	204	23.161
27	Cyclononasiloxane, octadecamethyl	4.34	666	23.421
28a	Alpha-amyrin	8.38	426	23.692
28b	Alpha-amyrin	5.91	426	24.237
29	Cyclononasiloxane, octadecamethyl	5.34	666	24.433
30	1-Octadecanesulphonyl chloride	4.17	352	24.706
31	Cyclononasiloxane, octadecamethyl	7.24	666	25.622
32	2-methylhexacosane	2.16	380	26.633

CONCLUSION

The leaf parts of *Momordica angustisepala* have been investigated in this research and preliminary phytochemical screening of the crude extracts shows the presence of bioactive compounds -that are of medicinal uses- such as tannins, glycosides, flavonoids, saponin, phenolics, terpenoids, steroids and anthraquinone. Antimicrobial activity of crude extracts from the two plants against all the test bacteria and fungi was found to be interesting at moderate to high concentration which justifies the ethnomedicinal uses of the plants for treating some diseases attributed to bacteria and fungi. Meanwhile, not all the test organisms (bacteria and fungi) were inhibited by the plant's extracts at these concentrations. For example, n-hexane and methanol extracts of *M. angustisepala* failed to inhibit the growths of some test bacteria. The GC-MS reveals various peaks of bioactive compounds of which the activities of the plants' extracts against bacteria and fungi, as well as their activities against free radicals, may be attributed. The most prominent compound with probable synergistic effect with all other compounds present in smaller quantities in the extracts proffers an explanation to the activity of the whole plants against the test organisms (bacteria and fungi) or free radical (DPPH).

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