Tropical Journal of Pharmaceutical Research May 2015; 14(5): 823-830
ISSN: 1596-5996 (print); 1596-9827 (electronic)
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Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v14i5.12

Original Research Article

Hypotensive Activity of *Moringa oleifera* Lam (Moringaceae) Root Extracts and its Volatile Constituents

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Received: 26 January 2015 *Revised accepted: 24 April 2015*

Abstract

Purpose: *To explore the hypotensive activity and chemical composition of Moringa oleifera Lam (Moringaceae) roots.*
Methods: *The fresh roots of M. oleifera was cut into small pieces and successively extracted with petroleum ether (PE) and dichloromethane (DC). PE extract was further divided into MRP and MRP -1. DC extract showed a thick mass during evaporation which was separated as MRDC - IN. The mother liquor left was divided into MRDC and MRDC -1. All residues were analyzed by gas chromatography-mass spectroscopy (GC-MS) using ZB-5 column. Identification of each extract and fraction was based on comparison of their retention indices (RI), by co-injecting authentic compounds, as well as by comparing literature data available in NIST Standard Reference Database. Hypotensive activity was determined on urethane-anesthetized normotensive Sprague Dawly rats.*
Results: *Petroleum ether (MRP) and dichloromethane (MRDC) extracts of M. oleifera roots showed 50.06 ± 3.48 and 48.16 ± 1.79 % fall in mean arterial blood pressure (MABP), respectively, at a dose of 30 mg/kg ($p < 0.01$ and $p < 0.05$, respectively) compared with control. GC-MS analysis of MRP and MRDC extracts and fractions resulted in the identification of seventy four (74) compounds. Methyl hexadecanoate (**7**, 20.3 %) , stigmastan - 3, 5, diene (**24**, 19.32 %), methyl 14-hydroxy-5-tetradecenoate (**9**, 19.22 %), 1 , 11 diphenyl undecane (**47**, 18.78 %) and cyclopentanyl hexadecane (**39**, 14.44 %) were the major constituents among the various hydrocarbons, fatty acids, esters, alcohols, aldehydes, isothiocyanate, aromatics, steroids, terphenyl and sulphur-containing compounds.*
Conclusion: *The findings reveal the hypotensive potential of M. oleifera roots and the presence of specific hydrocarbons, fatty acid esters, thioureides, steroids and isothiocyanates in active fractions. Further study is required to determine the suitability of the plant as an antihypertensive remedy.*

Keywords: *Moringa oleifera, Methyl hexadecanoate, Methyl 14-hydroxy-5-tetradecenoate, Petroleum ether, Stigmastan - 3, 5, diene, Cyclopentanyl hexadecane*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Moringa oleifera, Lam is one of the best known and widely utilized specie among the thirteen known species of the monogeneric family Moringaceae. It is native to Pakistan and India, and widely cultivated throughout the world [1,2]. Due to its implausible dietetic and therapeutical values, it is known as a “Miracle tree” and has been declared as complete and natural nutrition for the tropics and famine hit areas [3]. It is a prolific producer of diverse secondary metabolites including glycosides preferentially rhamnosides of carbamates, thiocarbamates,

nitriles, benzyl glucosinolates, thiocyanates, isothiocyanates and oxazolidine-2-thiones. Pharmacological screening of its leaf extracts exhibited hypotensive [4], cardioprotective [5], hepatoprotective [6], antidiabetic [7] and antiulcerogenic activities [8]. The pods are hypotensive [9] while seeds showed antibacterial [10], antitumor [11], anti-inflammatory and antispasmodic activities [12]. Roots were able to depress the central nervous system by producing analgesia and potentiate the analgesic effect of morphine [13]. Aurantiamide acetate and 1,3 dibenzyl urea isolated from roots showed significant anti inflammatory, antiarthritic and analgesic activity mediated through TNF- alpha, interleukin-2 and cytokines inhibition [14].

In recent decades therapeutic preparations like Septilin for respiratory tract infection, Rumalaya for arthralgia and Pro-lacta of *M. oleifera* have been marketed [15]. This paper reports the hypotensive evaluation and GC-MS analysis of *M. oleifera* roots constituents detected in winter, which are markedly different from those analyzed during summer [16]. Earlier volatile constituents from leaves [17], flowers [18] and pods [19] of *M. oleifera* have already been reported in literature.

EXPERIMENTAL

Plant material

Fresh roots of *M. oleifera* (10 kg) were collected in November 2007 from HEJ-ICCBS Garden University of Karachi, Karachi Pakistan. A voucher specimen (no. 66250 KUH) was deposited in the herbarium of Department of Botany, University of Karachi, where it was authenticated by Mr Abrar Hussain.

Extraction

Fresh roots of *M. oleifera* was cut into small pieces (1 - 2 inch) and successively extracted with petroleum ether (PE) and dichloromethane (DC) at room temperature for three days. PE extract was divided into two layers during evaporation. Both layers were separated and evaporated to residual masses MRP (upper layer, 3.14 g) and MRP - 1 (lower layer, 0.27 g). DC extract showed a thick mass settled at the bottom of round bottom flask during evaporation on rotavapour. It was separated, dried and weighed as MRDC - IN (2.25 g). Mother liquor left was further evaporated which when concentrated divided into two layers. Both layers were separated and evaporated to thick residues. Upper layer furnished MRDC (4.17 g) while lower layer gave MRDC - 1 (8.89 g).

Gas chromatography mass spectrometry

For GC-MS 6890 N Agilent gas chromatograph coupled with a JMS 600 H JEOL mass spectrometer was used. The compound mixture was separated on a fused silica capillary ZB-5 column, (30m x 0.32 mm) 0.22 µm film thickness in a temperature program from 50 to 260 ^{o}C with a rate of 4 ^{o}C min^{-1} with 3 min hold. The injector was set at 240 ^{o}C and the flow rate of helium carrier gas was 1 ml min^{-1}. The EI mode JMS 600 H JEOL mass spectrometer had ionization volt 70 eV, electron emission 100 ^{o}A. Sample was injected manually in split mode. Ratio of sample in split mode was 1:50. Identification of each extract and fractions were based on comparison of their retention indices (RI) calculated according to the Kovats formula, using n-alkanes (C9 – C33) (sigma –Aldrich, Germany) as standards under the same chromatographic conditions and in some cases by co-injection with authentic compounds as well as by following the characteristic mass fragmentation patterns of known compounds. Retention Indices were also compared with literature data available in National Institute of Standards and Technology Standard Reference Database. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Animals

Normotensive Sprague Dawley rats (both sex, 220 - 250g) were housed in the animal house of Dr. HMI Institute of Pharmacology and Herbal Sciences in appropriate cages at 21 - 23 ^{o}C. They were maintained at 12 h of an alternate light and dark cycle with free access to water and standard diet *ad libitum*. The maintenance and handling of laboratory animals and the experiments conducted follow the protocols based on internationally accepted standard guidelines of the Institutional Ethical Committee. The experimental procedures were performed according to international guidelines [20] and approved by the instutional ethical committee for handling laboratory animals (Ref no. HU/Dr.HMIIPHS/2013/11).

Hypotensive activity

Rats were anesthetized with urethane (1.2 gm/ kg i.p.). The trachea was exposed and cannulated with a polyethylene cannula to facilitate spontaneous respiration. Drugs were injected (0.2 - 0.25 ml) through a polyethylene cannula inserted into the extrajuglar vein followed by a saline flush (0.2 ml). The arterial blood pressure was recorded from the carotid

artery via an arterial cannula connected to research grade blood pressure transducer (Harvard, 60-3003) coupled with four channel Harvard oscillograph (Curvilinear, 50-9307)(UK). The temperature of the animals was maintained at 37 °C by over head heating lamp. The mean blood pressure was calculated as the sum of the diastolic blood pressure plus one–third pulse width. Changes in blood pressure were expressed as the percent of control obtained immediately before the administration of test substance. Acetylcholine (Ach) (Merck) at a dose of 1 µg/kg was used as positive control and atropine sulphate (0.1 mg/kg) (C. H. Beohringer Sohn Ingelheim Rhein, Germany) as muscarinic antagonist. MRP, MRP - 1 and MRDC were soluble in 5 % Tween 80 and others in normal saline.

Statistical analysis

Changes in blood pressure were compared using Students t-test (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY 2010). $P < 0.05$ was considered to be significant.

RESULTS

GC-MS (vide Tables 1-2) of MRP showed the presence of twenty nine compounds including five aromatics (**1**-**4**, **14**), two diterpenes (**11**, **12**), an isothiocyanate (**21**), a steroid (**24**) and six thioureido polymers (**13**, **15**, **17**, **19**, **22**, **23**). MRP-1 indicated eight compounds specifically isocyanate (**3**), isothiocyanate (**21**), thioureido (**23**), pyridine (**27**) and sesquiterpene (**28**). Analysis of MRDC - IN revealed eleven constituents, particularly long chain aromatic isothiocyanate (**21**), an aromatic urea (**30**) and three steroids (**24**, **31** and **32**). MRDC (Tables 1-4) showed the presence of eighteen compounds out of which six (**33**, **36**, **40**, **45**, **46** and **47**) contained both aromatic and aliphatic moieties. GC-MS study of MRDC - 1 detected thirty eight compounds together with fourteen aromatics (**3**, **10**, **33**, **46**, **48** - **53**, **58**, **65**, **67**, **71**), one lactone (**56**), one ketone (**62**) and four aldehydes (**63**, **66**, **68**, **70**).

Intravenous administration of extracts in anesthetized rats showed changes in systolic, diastolic and mean arterial blood pressure (MABP). Intravenous injection of positive control Ach at the dose of (1 µg/kg) showed (30.63 ± 3.5 %) fall in MABP while that of normal saline (0.9 % NaCl) was insignificant. Hypotensive evaluation of MRP showed significant fall in MABP at 3 mg/kg (41.84 ± 4.74 %, $p < 0.01$) and 30 mg/kg (50.06 ± 3.48 %, $p < 0.01$).

Hypotensive effect was comparable at both doses however; duration of activity was increased with dose increments (60 sec and 120 sec) at 3 and 30 mg/kg respectively. MRP - 1 displayed dose dependent hypotensive effect at 3 mg/kg (26.2 ± 5.06 %, $p < 0.05$) and 30 mg/kg (42.58 ± 1.21, $p < 0.05$) with same duration (41 s) at both doses. MRDC - IN and MRDC - 1 displayed significant ($p < 0.05$) dose-dependant fall in MABP (28.05 ± 3.04, 49.78 ± 3.83 and 22.51 ± 1.893, 38.57 ± 0.27 %, respectively at 3 and 30 mg/kg). Duration of action increased up to 25 min (1500 s) in case of MRDC -IN at higher dose while other doses of MRDC - IN and MRDC - 1 remained effective for about 34 s.

MRDC showed comparable behavior (46.61 ± 1.78 and 48.16± 1.79 %, $p < 0.05$) at both doses while duration of action was increased at 30 mg/kg (95.66 sec) as compared to 3 mg/kg (34 sec). MRP and MRP - 1 did not show any change in MABP when administered in rats pretreated with atropine sulphate, however, MRDC - IN, MRDC - 1 and MRDC remained impassive by atropine blockade.

DISCUSSION

GC-MS analysis of extracts and fractions culminated in the detection of eighty five compounds out of which seventy four have been identified. Not unexpectedly, hydrocarbons, and fatty acid esters were found as commonly occurring constituents. Hydrocarbons range from C17 to C33 as di, mono and unsubstituted compounds. 1, 11 diphenyl undecane (**47**, 18.78 %) and cyclopentanyl hexadecane (**39**, 14.44 %) were major hydrocarbon of MRDC. Among fatty acid esters, methyl esters dominate and ranges from tetradecanoate to tetracosanoate. Methyl hexadecanoate (**7**, 20.3 %) in MRDC and methyl 14-hydroxy-5-tetradecenoate (**9**, 19.22 %) in MRP - 1 were main esters. Isothiocyanates have been detected in all extracts except MRDC while halogenated derivatives were identified only in MRDC and MRDC – 1.

The characteristic feature of MRP is the presence of new and unique thioureido polymers (**14**, **15**, **17**, **19**, **22**, **23**) that make approximately 19 % of extract. They exhibited several mass fragments with characteristic difference of 74 amu indicating the possible loss of $(\text{-NH-CS-NH})^{+}$ from corresponding molecular fragments/ molecular ion peak. Steroids (approximately 37

Table 1: Volatile constituents (**1-27**) of various extracts of *Moringa oleifera* roots

S/N	Compounds[g]	Mol. formula (M.w)	RI[c]	RI[l]	Basis of identification	Extract	Content (%)
1	Benzaldehylde[c] (**1**)	C_7H_6O (106)	958	961	MS,RI	MRP	0.17
2	2-Amyl furan[c] (**2**)	$C_9H_{14}O$ (138)	987	1001	MS,RI	MRP	0.32
3	*p*-Tolyl isocyanate[a,b] (**3**)	C_8H_9NO (133)	1007	-	MS	MRP MRP-1 MRDC-1	0.78 7.76 0.21
4	Benzamide [c] (**4**)	C_7H_7ON (121)	1342	1344	MS,RI	MRP	0.46
5	Octadecane [c] (**5**)	$C_{18}H_{38}$ (254)	1800	1800	MS, RI	MRP MRDC	0.52 1.87
6	Nanodecane [b,c] (**6**)	$C_{19}H_{40}$ (268)	1900	1900	MS, RI	MRP MRDC-1	0.81 0.44
7	Methyl hexadecanoate [b,c] (**7**)	$C_{17}H_{34}O_2$ (270)	1935	1933	MS, RI	MRP MRDC - IN MRDC MRDC-1	3.31 3.18 20.3 7.80
8	Ethyl hexadecanoate [b,c] (**8**)	$C_{18}H_{30}O_2$ (284)	2013	2026	MS, RI	MRP MRDC MRDC-1	2.05 4.86 1.56
9	Methyl 14-hydroxy-5-tetradecenoate [d,e] (**9**)	$C_{15}H_{28}O_3$ (256)	2135	2130	RI	MRP MRP-1	8.89 19.22
10	(*Z*)-11-Eicosenoic acid [b,c] (**10**)	$C_{20}H_{38}O_2$ (310)	2286	2300	MS, RI	MRP MRDC-1	3.01 2.09
11	Abieta-9(11),8(14),12-trien-12-ol (*trans*) [d,e] (**11**)	$C_{20}H_{30}O$ (286)	2324	2325	MS, RI	MRP	3.48
12	Abieta-8,11,13-trien-7-one[d,e] (**12**)	$C_{20}H_{28}O$ (284)	2341	2315	MS, RI	MRP	3.07
13	Hepta thioureido- bis -1,1'-methylene amine [e] (**13**)	$C_9H_{22}N_{16}S_7$ (578)	2370	-	MS	MRP	3.56
14	Ethene 1,1'-bis–*p*-toulenyl sulfide [e] (**14**)	$C_{16}H_{16}S_2$ (272)	2414	-	MS	MRP MRDC-1	2.90 1.54
15	Octa thioureido- bis-1,1'-methylene amine [e] (**15**)	$C_{10}H_{24}N_{18}S_8$ (652)	2517	-	MS	MRP	4.05
16	Unidentified	-	2523		-	MRP	2.78
17	3,7-Dimethyl pentacosane [d,e] (**16**)	$C_{27}H_{56}$ (380)	2612	2608	MS,RI	MRP	1.77
18	Octa thioureido- bis -1,1'-methane [e] (**17**)	$C_{10}H_{22}N_{16}S_8$ (622)	2667	-	MS	MRP	2.03
19	Tertracosanoic acid [b,c] (**18**)	$C_{24}H_{48}O_2$ (368)	2725	2685	MS,RI	MRP	2.45
20	Hepta thioureido-bis-1,1'-thioamide [e] (**19**)	$C_9H_{18}N_{16}S_9$ (638)	2778	-	MS	MRP	2.88
21	3-Methyl heptacosane[d,e] (**20**)	$C_{28}H_{58}$ (394)	2764	2773	MS,RI	MRP	1.92
22	15- Phenyl pentadecanyl isothiocyanate [e] (**21**)	$C_{22}H_{35}NS$ (345)	2930	-	-	MRP MRP-1 MRDC - IN	4.10 9.40 6.36
23	Octa thioureido 1-hydroxy-1,1' amino methylene [e] (**22**)	$C_{10}H_{24}N_{18}S_8O$ (668)	3017	-	MS	MRP	2.89
24	Unidentified	-	3020	-	-	MRP	2.46

[a]Mass spectra were compared with literature fragmentation pattern given in National Institute of Standards and Technology *Standard Reference Database Number 69; [b]Mass and Retention index comparable with standard compound injected under similar condition; [c]Mass and Retention index comparable with values given in literature available in NIST database; [d]Retention index match with literature available in NIST database; [e]Compound tentatively identified according to observed mass fragmentation pattern; [f]Retention index value match with non equivalent column; [g]order of elution is given on column (ZB-5)*

Table 2: Volatile constituents (**25-46**) of various extracts of *Moringa oleifera* roots

S/N	Compounds[g]	Mol. formula (M.w)	RI[c]	RI[l]	Basis of identification	Extract	Content (%)
25	Unidentified	-	3047	-	-	MRP	2.47
26	Benzyl hexathioureido propane [e] (**23**)	$C_{16}H_{26}N_{12}S_6$ (578)	3070	-	MS	MRP MRP-1	3.19 7.14
27	Stigmastan-3,5-diene[d,e] (**24**)	$C_{29}H_{48}$ (396)	3036	3040	MS, RI	MRP MRDC-IN	5.66 19.32
28	3,7-Dimethyl triacontane [d,e] (**25**)	$C_{32}H_{66}$ (450)	3113	3110	MS, RI	MRP	1.14
29	9,13-Dimethyl hentriacontane [d,e] (**26**)	$C_{33}H_{68}$ (464)	3161	3162	MS, RI	MRP	1.21
30	2,5 Diethyl pyridine [c] (**27**)	$C_9H_{13}N$ (135)	1393	1422	MS, RI	MRP-1	2.42
31	1-β-Acetoxy furano-3-eudesmene [d,e] (**28**)	$C_{17}H_{24}O_3$ (276)	1978	1978	MS, RI	MRP-1	4.80
32	1,10 Diphenyl decane [e] (**29**)	$C_{22}H_{30}$ (294)	2581	-	MS	MRP-1	8.62
33	Unidentified	-	3058	-	-	MRP-1	6.94
34	Unidentified	-	2165	-	-	MRDC-IN	2.65
35	Unidentified	-	2427	-	-	MRDC-IN	1.88
36	Unidentified	-	2678	-	-	MRDC-IN	22.27
37	N,N- Dibenzyl undecanyl urea[e] (**30**)	$C_{26}H_{38}N_2O$ (394)	2930	-	MS	MRDC-IN	4.58
38	Ergosta-5,22-dien-3-ol, (3β,22E) [c] (**31**)	$C_{28}H_{46}O$ (398)	3017	3044	-	MRDC-IN	9.36
39	Unidentified	-	3033	-	-	MRDC-IN	10.69
40	Stigmastan -3,5,22 triene[e] (**32**)	$C_{29}H_{46}$ (394)	3044	2981	MS,RI	MRDC-IN	7.88
41	Unidentified	-	3056	-	-	MRDC-IN	7.04
42	*o*-Amino butyl benzene[c] (**33**)	$C_{10}H_{15}N$ (149)	1427	1390	MS, RI	MRDC MRDC-1	2.43 1.59
43	2-Methyl hexadecane [d] (**34**)	$C_{17}H_{34}$ (240)	1674	1666	MS, RI	MRDC	0.95
44	Methyl tetradecanoate [c] (**35**)	$C_{15}H_{30}O_2$ (242)	1708	1706	MS,RI	MRDC	1.08
45	1-3 Dibenzyl-3-ethyl urea[e](**36**)	$C_{17}H_{20}N_2O$ (268)	1969	-	MS	MRDC	2.51
46	Methyl (*E*)-9-hexadecenoate [c] (**37**)	$C_{17}H_{32}O_2$ (268)	1924	1912	MS, RI	MRDC	2.961

[a]Mass spectra were compared with literature fragmentation pattern given in National Institute of Standards and Technology *Standard Reference Database Number 69; [b]Mass and Retention index comparable with standard compound injected under similar condition; [c]Mass and Retention index comparable with values given in literature available in NIST database; [d]Retention index match with literature available in NIST database; [e]Compound tentatively identified according to observed mass fragmentation pattern; [f]Retention index value match with non equivalent column; [g]order of elution is given on column (ZB-5)*

Table 3: Volatile constituents (**47-68**) of various extracts of *Moringa oleifera* roots

S/N	Compounds[g]	Mol. formula (M.w)	RI[c]	RI[l]	Basis of identification	Extract	Content (%)
47	(E)-6-Octadecenoic acid [d,e] (**38**)	$C_{18}H_{34}O_2$ (282)	2085	2073	MS, RI	MRDC	6.07
48	Cyclopentanyl hexadecane [d,e] (**39**)	$C_{21}H_{42}$ (294)	2182	2167	RI	MRDC	14.44
49	Decyl-3-chlorobenzoate [d,e] (**40**)	$C_{17}H_{25}ClO_2$ (296)	2189	2173	MS, RI	MRDC	12.0
50	Methyl (*Z*)-13-Octadecenoate [d,e] (**41**)	$C_{19}H_{36}O_2$ (296)	2151	2126	MS, RI	MRDC MRDC-1	9.02 4.97
51	Propyl heptadecanoate [d,e] (**42**)	$C_{20}H_{40}O_2$ (312)	2221	2193	RI	MRDC	4.10
52	(*Z*)-13-Eicosenoic acid [d,e] (**43**)	$C_{20}H_{38}O_2$ (310)	2312	2365	MS, RI	MRDC	7.24
53	12-Methyl tetracosane [d,e] (**44**)	$C_{25}H_{52}$ (352)	2439	2434	MS,RI	MRDC	5.00
54	N,N-Dibenzyl-3N- pentyl thiourea[e] (**45**)	$C_{20}H_{26}N_2S$ (326)	2455	-	MS	MRDC	5.69
55	Dibenzyl phthalate [c] (**46**)	$C_{22}H_{18}O_4$ (346)	2647	2690	MS,RI	MRDC MRDC-1	5.77 1.37
56	1,11 Diphenyl undecane [e] (**47**)	$C_{23}H_{32}$ (308)	3017	-	MS	MRDC	18.78
57	1-Chloro-2-methyl benzene[c] (**48**)	C_7H_7Cl (126)	913	955	RI	MRDC-1	0.06
58	Benzyl thiol [c] (**49**)	C_7H_8S (124)	969	1067	MS	MRDC-1	0.13
59	Benzyl isothiocyanate[c] (**50**)	C_8H_7NS (149)	1281	1320	MS, RI	MRDC-1	0.21
60	Methyl 3,3-diphenyl propanoate [e] (**51**)	$C_{16}H_{16}O_2$ (240)	1692	-	MS	MRDC- 1	0.13
61	4 (2'-Amino) ethyl benzyl isocyanate[e] (**52**)	$C_{10}H_{12}N_2O$ (176)	1781	-	MS	MRDC-1	0.35
62	Di-3-phenyl propyl ether [e] (**53**)	$C_{18}H_{22}O$ (254)	1789	-	MS	MRDC-1	0.48
63	Tetradecanoic acid [b,c] (**54**)	$C_{14}H_{28}O_2$ (228)	1745	1761	MS, RI	MRDC-1	0.27
64	Methyl (Z)-9-hexadecenoate [c] (**55**)	$C_{17}H_{32}O_2$ (268)	1922	1912	MS ,RI	MRDC-1	0.95
65	Tetrahydro-6-undecanyl-2H-pyran-2-one[c] (**56**)	$C_{16}H_{30}O_2$ (254)	2070	2070	MS ,RI	MRDC-1	1.053
66	Methyl heptadecanoate[c] (**57**)	$C_{18}H_{36}O_2$ (284)	2083	2028	MS, RI	MRDC-1	1.04
67	(*E*)-3,7-Dimethyl octa-2,6-dienyl-3-chlorobenzoate [c] (**58**)	$C_{17}H_{21}Cl\ O_2$ (292)	2176	2150	MS, RI	MRDC-1	4.65
68	Methyl (*Z*)-9-octadecenoate[d] (**59**)	$C_{19}H_{36}O_2$ (296)	2146	2106	MS, RI	MRDC-1	3.47

[a]Mass spectra were compared with literature fragmentation pattern given in National Institute of Standards and Technology *Standard Reference Database Number 69; [b]Mass and Retention index comparable with standard compound injected under similar condition; [c]Mass and Retention index comparable with values given in literature available in NIST database; [d]Retention index match with literature available in NIST database; [e]Compound tentatively identified according to observed mass fragmentation pattern; [f]Retention index value match with non equivalent column; [g]order of elution is given on column (ZB-5)*

Table 4: Volatile constituents (**69-85**) of various extracts of *Moringa oleifera* roots

S/N	Compounds[g]	Mol. formula (M.w)	RI[c]	RI[l]	Basis of identification	Extract	Content (%)
69	Methyl octadecanoate[d] (**60**)	$C_{19}H_{38}O_2$ (298)	2158	2139	MS, RI	MRDC-1	1.67
70	Methyl (*Z,Z*)-11,14-eicosadienoate[d] (**61**)	$C_{21}H_{38}O_2$ (322)	2250	2279	MS,RI	MRDC-1	0.09
71	2-Docosanone [c] (**62**)	$C_{22}H_{44}O$ (324)	2408	2410	MS, RI	MRDC-1	1.71
72	Tricosanal [c] (**63**)	$C_{23}H_{46}O$ (338)	2510	2530	MS, RI	MRDC-1	1.73
73	3,11-Dimethyl pentacosane [d] (64)	$C_{27}H_{56}$ (380)	2602	2607	MS, RI	MRDC-1	1.87
74	1-(*p*-Benzyl) phenyl -7-phenyl-1-heptene [e] (**65**)	$C_{27}H_{30}$ (354)	2652	-	MS	MRDC-1	1.09
75	Pentacosanal [c] (**66**)	$C_{25}H_{50}O$ (366)	2719	2733	MS, RI	MRDC-1	2.19
76	*1-p*-Toluenyl-2-fluoro,4-(3'-methyl-4' phenyl) phenyl -5– methyl benzene [e] (**67**)	$C_{27}H_{25}F$ (368)	2745	-	MS	MRDC-1	2.23
77	Hexacosanal [c] (**68**)	$C_{26}H_{52}O$ (380)	2789	2830	MS	MRDC-1	2.47
78	Methyl tetracosanoate [e] (**69**)	$C_{25}H_{50}O_2$ (382)	2833		MS	MRDC-1	1.04
79	Heptacosanal [c] (**70**)	$C_{27}H_{54}O$ (394)	2918	2930	MS	MRDC-1	2.17
80	Unidentified	-	2928	-	-	MRDC-1	3.21
81	1,4 -Ditoluenyl 2- chloro–5-methyl -2,5 cyclohexadiene [e] (**71**)	$C_{22}H_{23}Cl$ (322)	3019	-	MS	MRDC-1	9.09
82	(*Z,Z*)-9,18-Hentriacontadiene[f] (**72**)	$C_{31}H_{60}$ (432)	3070	3055	RI	MRDC-1	2.24
83	2-Methyl triacontane [f] (**73**)	$C_{31}H_{64}$ (436)	3064	3065	MS, RI	MRDC-1	2.06
84	13-Methyl hentriacontane [d,e] (**74**)	$C_{32}H_{66}$ (450)	3110	3130	MS, RI	MRDC-1	0.78
85	Unidentified	-	3154	-	-	MRDC-1	0.71

[a]Mass spectra were compared with literature fragmentation pattern given in National Institute of Standards and Technology *Standard Reference Database Number 69; [b]Mass and Retention index comparable with standard compound injected under similar condition; [c]Mass and Retention index comparable with values given in literature available in NIST database; [d]Retention index match with literature available in NIST database; [e]Compound tentatively identified according to observed mass fragmentation pattern; [f]Retention index value match with non equivalent column; [g]order of elution is given on column (ZB-5)*

%) were observed as main class of compounds in MRDC - IN. MRDC – 1 showed presence of most diversified constituents including aldehydes, isocyanates, ketone and lactone along with other mentioned compounds.

All extracts and fractions examined showed significant hypotensive activity, however, as far as mode of action is concerned, PE and DC – extracts have opposite behavior. MRP and MRP - 1 mediate through muscarinic receptors as both did not show any change in MABP in atropine pretreated animals. Stimulation of muscarinic receptors by MRP and MRP – 1 may cause the release of nitric oxide or endothelium derived relaxing factors (EDRF) that diffuse in smooth muscle cells and initiate immediate decrease in MABP. MRDC - IN, MRDC - 1 and MRDC appear to espouse pathways other than cholinergic. Further research for toxicology and exact mode of action is required.

CONCLUSION

The hypotensive activity of non-polar extracts and fractions of *Moringa oleifera* roots and their chemical composition through GC-MS have been established. However, further studies are needed to ascertain its bioactivity, especially in a hypertensive model. This will help to establish the complementary effect of these components and their suitability as an antihypertensive remedy.

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