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

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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, thioureas, 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, Stigmasan - 3, 5, diene, Cyclopentanyl hexadecane

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 preparatons 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 °C with a rate of 4 °C min⁻¹ with 3 min hold. The injector was set at 240 °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 °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 °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 institutional ethical committee for handling laboratory animals (Ref no. HU/Dr.HMIIPHIS/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. Boehringer 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 isocyante (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 (-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* | Mol. formula (M.w) | Ret* | Ret† | Basis of identification | Extract | Content (%) |
|-----|------------|--------------------|------|------|-------------------------|---------|-------------|
| 1   | Benzaldehyde c (1) | C₆H₅O (106) | 958 | 961 | MS, RI | MRP | 0.17 |
| 2   | 2-Amyl furan c (2) | C₉H₁₂O (138) | 987 | 1001 | MS, RI | MRP | 0.32 |
| 3   | p-Tolyl isocyanate a,b (3) | C₆H₅NO (133) | 1007 | - | MS | MRP | 0.78 |
| 4   | Benzamide c (4) | C₈H₁₂N₄O (121) | 1342 | 1344 | MS, RI | MRP | 0.46 |
| 5   | Octadecane c (5) | C₁₈H₃₈ (254) | 1800 | 1800 | MS, RI | MRP | 0.52 |
| 6   | Nanodecane b,c (6) | C₁₉H₄₀ (268) | 1900 | 1900 | MS, RI | MRP | 0.81 |
| 7   | Methyl hexadecanoate b,c (7) | C₁₇H₃₈O₂ (270) | 1935 | 1933 | MS, RI | MRP | 0.44 |
| 8   | Ethyl hexadecanoate b,c (8) | C₁₈H₃₈O₂ (284) | 2013 | 2026 | MS, RI | MRP | 3.31 |
| 9   | Methyl 14-hydroxy-5-tetradecenoate d,e (9) | C₁₄H₂₃O₃ (256) | 2135 | 2130 | RI | MRP | 2.05 |
| 10  | (Z)-11-Eicosenoic acid b,c (10) | C₁₉H₃₀O₂ (310) | 2286 | 2300 | MS, RI | MRP | 4.86 |
| 11  | Abiet-9(11),8(14),12-trien-12-ol (trans) d,e (11) | C₂₀H₃₂O (286) | 2324 | 2325 | MS, RI | MRP | 1.56 |
| 12  | Abiet-8,11,13-tren-7-one b,c (12) | C₂₁H₃₄O (284) | 2341 | 2315 | MS, RI | MRP | 19.22 |
| 13  | Hepta thioureido- bis -1,1'-methylene amine b,c (13) | C₁₇H₁₉N₂S₂ (578) | 2370 | - | MS | MRP | 3.01 |
| 14  | Ethene 1,1'-bis-p-toulenyl sulfide b,c (14) | C₁₈H₃₂S₂ (272) | 2414 | - | MS | MRP | 2.09 |
| 15  | Octa thioureido- bis -1,1'-methylene amine b,c (15) | C₁₉H₃₄N₂₁S₈ (652) | 2517 | - | MS | MRP | 3.48 |
| 16  | Unidentified | - | 2523 | - | MS,RRI | MRP | 2.90 |
| 17  | 3,7-Dimethyl pentacosane b,c (16) | C₂₇H₅₄ (380) | 2612 | 2608 | MS,RI | MRP | 1.54 |
| 18  | Octa thioureido- bis -1,1'-methylene b,c (17) | C₂₁H₁₉N₂₁S₈ (622) | 2667 | - | MS | MRP | 4.05 |
| 19  | Tertracosanolic acid b,c (18) | C₂₃H₄₀O₂ (368) | 2725 | 2685 | MS,RI | MRP | 2.78 |
| 20  | Hepta thioureido-bis -1,1'-thioamide b,c (19) | C₂₁H₂₁N₂₂S₂ (638) | 2777 | - | MS | MRP | 1.77 |
| 21  | 3-Methyl heptacosane b,c (20) | C₂₃H₄₈ (394) | 2764 | 2773 | MS,RI | MRP | 2.03 |
| 22  | 15-Phenyl pentadecanyl isoctiocyanoate b,c (21) | C₂₀H₂₃NS (345) | 2930 | - | - | MRP | 2.88 |
| 23  | Octa thioureido 1-hydroxy-1,1' amino methylene b,c (22) | C₁₀H₂₃N₂₁S₂O (668) | 3017 | - | MS | MRP | 4.10 |
| 24  | Unidentified | - | 3020 | - | - | MRP | 9.40 |

*Mass spectra were compared with literature fragmentation pattern given in National Institute of Standards and Technology Standard Reference Database Number 69; †Mass and Retention index comparable with standard compound injected under similar condition; ‡Mass and Retention index comparable with values given in literature available in NIST database; §Retention index match with literature available in NIST database; ¶Compound tentatively identified according to observed mass fragmentation pattern; ‡‡Retention index value match with non equivalent column; *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<sup>a</sup> | Mol. formula (M.w) | RI<sup>b</sup> | RI<sup>c</sup> | Basis of identification | Extract | Content (%) |
|-----|------------------------|--------------------|---------------|---------------|-------------------------|---------|-------------|
| 25  | Unidentified           | -                  | 3047          | -             | -                       | MRP     | 2.47        |
| 26  | Benzyl hexathioureido propane<sup>a</sup> (23) | C<sub>16</sub>H<sub>26</sub>N<sub>12</sub>S<sub>6</sub> (578) | 3070          | -             | MS                      | MRP     | 3.19        |
| 27  | Stigmasteran-3,5-diene<sup>d,e</sup> (24) | C<sub>28</sub>H<sub>48</sub> (396) | 3036          | 3040          | MS, RI                  | MRP     | 5.66        |
| 28  | 3,7-Dimethyl triacontane<sup>d,e</sup> (25) | C<sub>32</sub>H<sub>66</sub> (450) | 3113          | 3110          | MS, RI                  | MRP     | 1.14        |
| 29  | 9,13-Dimethyl hentriacontane<sup>d,e</sup> (26) | C<sub>33</sub>H<sub>68</sub> (464) | 3161          | 3162          | MS, RI                  | MRP     | 1.21        |
| 30  | 2,5 Diethyl pyridine<sup>e</sup> (27) | C<sub>9</sub>H<sub>13</sub>N (135) | 1393          | 1422          | MS, RI                  | MRP     | 2.42        |
| 31  | 1-β-Acetoxo furano-3-eudesmene<sup>d,e</sup> (28) | C<sub>17</sub>H<sub>24</sub>O<sub>3</sub> (276) | 1978          | 1978          | MS, RI                  | MRP     | 4.80        |
| 32  | 1,10 Diphenyl decane<sup>e</sup> (29) | C<sub>22</sub>H<sub>30</sub> (294) | 2581          | -             | MS                      | MRP     | 8.62        |
| 33  | Unidentified           | -                  | 3058          | -             | -                       | MRP     | 6.94        |
| 34  | Unidentified           | -                  | 2165          | -             | -                       | MRP     | 2.65        |
| 35  | Unidentified           | -                  | 2427          | -             | -                       | MRP     | 1.88        |
| 36  | Unidentified           | -                  | 2678          | -             | -                       | MRP     | 22.27       |
| 37  | N,N- Dibenzyl undecanyl urea<sup>e</sup> (30) | C<sub>28</sub>H<sub>38</sub>N<sub>2</sub>O (394) | 2930          | -             | MS                      | MRP     | 4.58        |
| 38  | Ergosta-5,22-dien-3-ol, (38,22E)<sup>e</sup> (31) | C<sub>28</sub>H<sub>46</sub>O (398) | 3017          | 3044          | -                       | MRP     | 9.36        |
| 39  | Unidentified           | -                  | 3033          | -             | -                       | MRP     | 10.69       |
| 40  | Stigmasteran -3,5,22 triene<sup>e</sup> (32) | C<sub>28</sub>H<sub>46</sub> (394) | 3044          | 2981          | MS, RI                  | MRP     | 7.88        |
| 41  | Unidentified           | -                  | 3056          | -             | -                       | MRP     | 7.04        |
| 42  | α-Amino butyl benzene<sup>e</sup> (33) | C<sub>13</sub>H<sub>15</sub>N (149) | 1427          | 1390          | MS, RI                  | MRP     | 2.43        |
| 43  | 2-Methyl hexadecane<sup>a</sup> (34) | C<sub>17</sub>H<sub>34</sub> (240) | 1674          | 1666          | MS, RI                  | MRP     | 0.95        |
| 44  | Methyl tetradecanoate<sup>e</sup> (35) | C<sub>13</sub>H<sub>26</sub>O<sub>2</sub> (242) | 1708          | 1706          | MS, RI                  | MRP     | 1.08        |
| 45  | 1-3 Dibenzyl-3-ethyl urea<sup>e</sup> (36) | C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O (268) | 1969          | -             | MS                      | MRP     | 2.51        |
| 46  | Methyl (E)-9-hexadecenoate<sup>e</sup> (37) | C<sub>17</sub>H<sub>22</sub>O (268) | 1924          | 1912          | MS, RI                  | MRP     | 2.96        |

<sup>a</sup>Mass spectra were compared with literature fragmentation pattern given in National Institute of Standards and Technology Standard Reference Database Number 69; <sup>b</sup>Mass and Retention index comparable with standard compound injected under similar condition; <sup>c</sup>Mass and Retention index comparable with values given in literature available in NIST database; <sup>d</sup>Retention index match with literature available in NIST database; <sup>e</sup>Compound tentatively identified according to observed mass fragmentation pattern; <sup>f</sup>Retention index value match with non equivalent column; <sup>g</sup>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\(^9\) | Mol. formula | Ret\(^a\) | Ret\(^b\) | Basis of identification | Extract | Content (%) |
|-----|--|-----------------|--------------|-------------|-----------------------|---------|-------------|
| 47  | (E)-6-Octadecenoic acid \(^{d,e}\) \((38)\) | C\(_{16}\)H\(_{22}\)O\(_2\) \((282)\) | 2085 | 2073 | MS, RI | MRDC | 6.07 |
| 48  | Cyclopentanylg 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\(_{16}\)H\(_{28}\)O\(_2\) \((296)\) | 2151 | 2126 | MS, RI | MRDC | 9.02 |
| 51  | Propyl heptadecanoate \(^{d,e}\) \((42)\) | C\(_{20}\)H\(_{32}\)O\(_2\) \((312)\) | 2221 | 2193 | RI | MRDC | 4.10 |
| 52  | (Z)-13-Eicosenoic acid \(^{d,e}\) \((43)\) | C\(_{22}\)H\(_{34}\)O\(_2\) \((310)\) | 2312 | 2365 | MS, RI | MRDC | 7.24 |
| 53  | 12-Methyl tetrasanoate \(^{d,e}\) \((44)\) | C\(_{22}\)H\(_{32}\) \((352)\) | 2439 | 2434 | MS, RI | MRDC | 5.00 |
| 54  | N,N-Dibenzy-3N-pentyl thiourea \(^{a}\) \((45)\) | C\(_{20}\)H\(_{38}\)N\(_2\)S \((326)\) | 2455 | - | MS | MRDC | 5.69 |
| 55  | Dibenzy phthalate \(^c\) \((46)\) | C\(_{22}\)H\(_{40}\)O\(_4\) \((346)\) | 2647 | 2690 | MS, RI | MRDC | 5.77 |
| 56  | 1,11 Diphenyl undecane \(^e\) \((47)\) | C\(_{23}\)H\(_{32}\) \((308)\) | 3017 | - | MS | MRDC | 18.78 |
| 57  | 1-Chloro-2-methyl benzene \(^e\) \((48)\) | C\(_{6}\)H\(_{4}\)Cl \((126)\) | 913 | 955 | RI | MRDC-1 | 0.06 |
| 58  | Benzyl thiol \(^c\) \((49)\) | C\(_{6}\)H\(_{5}\)S \((124)\) | 969 | 1067 | MS | MRDC-1 | 0.13 |
| 59  | Benzyl isothiocyanate \(^c\) \((50)\) | C\(_{6}\)H\(_{5}\)N\(_2\)S \((149)\) | 1281 | 1320 | MS, RI | MRDC-1 | 0.21 |
| 60  | Methyl 3,3-diphenyl propanoate \(^a\) \((51)\) | C\(_{16}\)H\(_{26}\)O\(_2\) \((240)\) | 1692 | - | MS | MRDC-1 | 0.13 |
| 61  | 4 (2'-Amino) ethyl benzyl isocyanate \(^c\) \((52)\) | C\(_{16}\)H\(_{22}\)N\(_2\)O \((176)\) | 1781 | - | MS | MRDC-1 | 0.35 |
| 62  | Di-3-phenyl propyl ether \(^e\) \((53)\) | C\(_{16}\)H\(_{22}\)O \((254)\) | 1789 | - | MS | MRDC-1 | 0.48 |
| 63  | Tetradecanoic acid \(^b,c\) \((54)\) | C\(_{16}\)H\(_{32}\)O\(_2\) \((282)\) | 1745 | 1761 | MS, RI | MRDC-1 | 0.27 |
| 64  | Methyl (Z)-9-hexadecenoate \(^c\) \((55)\) | C\(_{16}\)H\(_{22}\)O\(_2\) \((288)\) | 1922 | 1912 | MS, RI | MRDC-1 | 0.95 |
| 65  | Tetrahydoro-6-undecanly-2H-pyran-2-one \(^c\) \((56)\) | C\(_{18}\)H\(_{36}\)O\(_2\) \((284)\) | 2070 | 2070 | MS, RI | MRDC-1 | 1.053 |
| 66  | Methyl heptadecanoate \(^c\) \((57)\) | C\(_{18}\)H\(_{36}\)O\(_2\) \((294)\) | 2083 | 2028 | MS | MRDC-1 | 1.04 |
| 67  | (E)-3,7-Dimethyl octa-2,6-dienyl-3-chlorobenzoate \(^c\) \((58)\) | C\(_{17}\)H\(_{21}\)ClO\(_2\) \((292)\) | 2176 | 2150 | MS, RI | MRDC-1 | 4.65 |
| 68  | Methyl (Z)-9-octadecenoate \(^d\) \((59)\) | C\(_{18}\)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)
As mode of action is concerned, PE and DC significant hypotensive activity, however, a
All extracts and fractions examined showed along with other mentioned compounds.
aldehydes, isocyanates, ketone and lactone
most diversified constituents including
in MRDC
match with non equivalent column;
literature available in NIST database;
compound injected under similar condition;
Technology
Compound tentatively identified according to observed mass fragmentation pattern;
Mass spectra were compared with literature fragmentation pattern given in
Compounds
Z
Mol. formula (M.w)
RI\textsuperscript{a}
RI\textsuperscript{b}
Basis of identification
Extract
Content (%)
69 Methyl octadecanoate\textsuperscript{c} (60) C\textsubscript{28}H\textsubscript{56}O\textsubscript{2} (298) 2158 2139 MS, RI MRDC-1 1.67
70 Methyl (Z,Z)-11,14-ecicosadienoate\textsuperscript{c} (61) C\textsubscript{29}H\textsubscript{58}O\textsubscript{2} (322) 2250 2279 MS, RI MRDC-1 0.09
71 2-Docosanone \textsuperscript{e} (62) C\textsubscript{29}H\textsubscript{64}O (324) 2408 2410 MS, RI MRDC-1 1.71
72 Tricosanal \textsuperscript{c} (63) C\textsubscript{30}H\textsubscript{66}O (338) 2510 2530 MS, RI MRDC-1 1.73
73 3,11-Dimethyl pentacosane \textsuperscript{e} (64) C\textsubscript{31}H\textsubscript{68} (380) 2602 2607 MS, RI MRDC-1 1.87
74 1-(p-Benzyl) phenyl -7-phenyl-1-heptene \textsuperscript{c} (65) C\textsubscript{21}H\textsubscript{30} (354) 2652 - MS MRDC-1 1.09
75 Pentacosan-1 \textsuperscript{c} (66) C\textsubscript{30}H\textsubscript{62}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 \textsuperscript{c} (67) C\textsubscript{21}H\textsubscript{28}F (368) 2745 - MS MRDC-1 2.23
77 Hexacosanal \textsuperscript{c} (68) C\textsubscript{30}H\textsubscript{62}O (380) 2789 2830 MS MRDC-1 2.47
78 Methyl tetracosanoate \textsuperscript{e} (69) C\textsubscript{31}H\textsubscript{64}O\textsubscript{2} (382) 2833 MS MRDC-1 1.04
79 Heptacosanal \textsuperscript{c} (70) C\textsubscript{29}H\textsubscript{58}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 \textsuperscript{c} (71) C\textsubscript{27}H\textsubscript{25}Cl (322) 3019 - MS MRDC-1 9.09
82 (Z,Z)-9,18-Hentriacontadiene \textsuperscript{c} (72) C\textsubscript{31}H\textsubscript{60} (432) 3070 3055 RI MRDC-1 2.24
83 2-Methyl triacontane \textsuperscript{c} (73) C\textsubscript{31}H\textsubscript{64} (436) 3064 3065 MS, RI MRDC-1 2.06
84 13-Methyl hentriacontane \textsuperscript{c} (74) C\textsubscript{32}H\textsubscript{68} (450) 3110 3130 MS, RI MRDC-1 0.78
85 Unidentified - 3154 - - MRDC-1 0.71

\textsuperscript{a}Mass spectra were compared with literature fragmentation pattern given in National Institute of Standards and Technology Standard Reference Database Number 69; \textsuperscript{b}Mass and Retention index comparable with standard compound injected under similar condition; \textsuperscript{c}Mass and Retention index comparable with values given in literature available in NIST database; \textsuperscript{d}Retention index match with literature available in NIST database; \textsuperscript{e}Compound tentatively identified according to observed mass fragmentation pattern; \textsuperscript{f}Retention index value match with non equivalent column; \textsuperscript{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.

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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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