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Chemical Composition, Antibacterial and *in vitro* Anti-Inflammatory Potentials of Essential Oils from Different Plant Parts of *Moringa oleifera* Lam

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Abstract: The chemical constituents, antibacterial, protection against protein denaturation and membrane stabilization activities of essential oils from flowers, leaves, seeds, bark and roots of *M. oleifera* were investigated. Of the eight bacterial strains tested, only the growths of four strains were inhibited by the essential oils. The oils from the flowers and seeds were the most active with MIC: 1.25 mg/mL each against *P. vulgaris* and *K. pneumoniae* respectively. All tested concentrations of *M. oleifera* essential oils showed high inhibition of protein denaturation (IC_{50} 0.2 mg/mL) and high membrane stability (IC_{50}: < 0.1 mg/mL) compared to Diclofenac. The essential oils were dominated by eicosane (20.93%, 17.12% and 21.59%) in flowers, leaves and seeds respectively; naphthalene (18.40%) in bark and benzene isothiocyanato methyl (35.83%) in the roots. The results revealed that essential oils from different plant parts of *M. oleifera* could be explored as potential candidates with alternative or complementary potentials for combating drug resistant bacteria and inflammation.

Keywords: Antibacterial Activity, Bioactive Constituents, Essential Oil, Membrane Stability, *Moringa oleifera*, Protein Denaturation

Introduction

Moringa oleifera is an ancient tree that is traditionally renowned for its numerous nutritional and pharmacological properties. *M. oleifera* belongs to the *Moringaceae* family, with 12 other species and although originally from India, it can now be found growing world-wide (Fahey, 2005; Price, 2007).

Nutritionally, the leaves are rich sources of macro- and micro-nutrients. According to Fahey (2005) the leaves are richer in vitamins A and C, calcium, potassium and protein than most conventional sources. Traditionally, all the different parts of the plant are consumed as vegetables and several information of the nutritional properties have been reported (Maponga and Monera (2010; Patel *et al*., 2010; Amrutia *et al*., 2011). Pharmacologically, *M. oleifera* plant parts have been credited with therapeutic properties against hypertension, convulsions, diabetes, cancer, inflammation, liver diseases, gastric ulcers and skin diseases (Pandey, 2012; Bakre *et al*., 2013). The crude, supernatant, residue and dialyzed seed extract have antimicrobial action (Jabeen *et al*., 2008), while the aqueous and ethanol extracts of the seeds have also been reported to possess antibacterial effect (Vieira *et al*., 2010). In addition, essential oil components of Moringa leaf (Kuben and Roger, 2011; Moyo *et al*., 2013), seed (Chuang *et al*., 2007) and root (petroleum ether) extract (Mukunzi *et al*., 2011) have been reported.

Inflammation is the cellular response to injury; and a complex cascade of reactions including increased vascular permeability, protein denaturation and membrane alterations, as well as the accumulation of cells and exudates in irritated tissues to protect and prevent further damage (Umapathy *et al*., 2010; Padmanabhan and Jangle, 2012; Osman *et al*., 2016). Several models have been used for evaluating the anti-inflammatory potentials of plant extracts *in vivo* and *in vitro*. However, protein denaturation assay and erythrocyte membrane stability are the two mostly used assays for *in vitro* studies.

Denatured proteins lose their biological potency *in vivo* and become inflammatory mediators (Khan *et al*., 2015), which cause inflammation through the production of auto antigens and increased activity of macrophages

(Tatiya and Saluja, 2011). Inflammation can be prevented by stabilizing the lysosomal membrane, since damage to the membrane will release lysosomal enzymes that produce diverse disorders related inflammation. Erythrocytes membranes structurally resemble lysosomal membranes; therefore, preventing the breakdown of the erythrocyte membrane is also often used as a measure for estimating the anti-inflammatory potentials of plant extracts *in vitro* (Omale and Okafor, 2008).

Phytochemicals possessing anti-inflammatory properties have been used traditionally over time for the treatment of inflammatory conditions such as fevers, pain, migraine and arthritis. Subsequently, extracts from food and food products which have anti-inflammatory actions are of great interest (Yua *et al*., 2006).

Moringa oleifera essential oils have exhibited *in vitro* anti-oxidant, anti-microbial, anti-hypertensive, anti-cancer, anti-tubercular and anti-inflammatory activities (Mishra *et al*., 2011; Pandey, 2012; Bakre *et al*., 2013); and also contains many bioactive constituents (Chuang *et al*., 2007; Moyo *et al*., 2013). The continuous search for safe, cheap and affordable treatments for various ailments, especially those caused by resistant microbial pathogens and inflammation, has led to investigations for bioactive molecules from essential oils from different parts of many plant species. According to Vieira *et al*. (2010) multi-drug resistant microbial strains is an increasing health concern globally, which justifies the current search for novel antimicrobial and antiinflammatory agents.

Even though several reports on *Moringa oleifera* abound in literature, the constituents of the essential oil of the various parts, their antibacterial and anti-inflammatory potentials still remain unrecorded. Therefore, this study aimed at evaluating the composition, antibacterial and anti-inflammatory properties of essential oils from different parts of *Moringa oleifera*.

Materials and Methods

Plant Materials

Different parts of *Moringa oleifera* Lam. were collected in clean plastic containers from the University of Ilorin Moringa Plantation, Nigeria in January 2014. The parts were cleaned and oven-dried (40°C, 72 h), separately packed into clean ice-packed plastic containers, then transported to the MPED-RNA, UFH, South Africa; where specimens were deposited at the Giffen Herbarium of the University.

Extraction of Essential Oils

The Solvent Free Microwave Extractor was used. One hundred gram of each plant part was separately weighed into the reactor of an automatic Milestone DryDIST 2004, Bergamo, Italy, microwave apparatus and individually extracted without water or solvent.

Homogenous microwave energy distribution in the reactor was ensured with a microwave multimode reactor having twin magnetron (2800 W, 2450 MHz) and maximum delivery power of 1000 W in 10 W increments and a rotating microwave diffuser. A shielded Thermocouple (ATC-300) inserted directly into the corresponding container was used to monitor the temperature, while a feedback to the microwave power regulator, controlled the temperature (initial temperature of 20°C, increased at 7.5°C/min up to 100°C) before essential oil extraction for 10 min. The total extraction process was 30 min and the temperature decreased at a rate of 7.0°C/min until 30°C was reached. N-hexane (1.0 mL) was used to retain the extracted oil in the collector column. Yields were calculated and the oils stored in amber essential oil bottles at 4°C prior until needed.

Essential Oils Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) separation of the oils was carried out in an Agilent 7890B GC coupled to an Agilent 5977A mass selective detector and an Agilent ChemStation data system. The machine was equipped with a Zebron-5MS column [ZB-5MS 30m ×0.25 mm 5%-phenyl methyl polysiloxane capillary column, film thickness (0.25 μm)]; injector temperature 280°C and source temperature 280°C. Initial oven temperature was 70°C for 15 min; increased at 3 min to 120°C, ramp at 10°C/min to 180°C, then ramp at 20°C/min to 270°C for 3 minutes. Helium at a flow rate of 2 mL/min was used as the carrier gas. The sample injected into the machine was 1 μL using splitless injection technique. The various constituents were identified by comparing their mass spectra, retention indices using commercial libraries (NIST2014 GC-MS) and with those available in literature (Adams, 2007) as well as using the C_8-C_{20} alkane standards.

Antimicrobial Assays

Microbial Strains and Media

The essential oil samples were screened against *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* ATCC 19582, *Serratia marcescens* ATCC 9986, *Shigella flexneri* KZN, *Proteus vulgaris*, *Klebsiella pneumonia* ATCC 4352 and *Staphylococcus aureus*. Sabouraud Dextrose Agar (SDA) and Broth (SDB) were prepared following manufacturer's instructions. The agar was dissolved in de-ionised water and sterilized by autoclaving for 15 min at 121°C. Bacterial strains were kept on SDA plates at 4°C, inoculums were prepared by diluting cell mass in 0.85% sodium chloride solution, adjusted to 0.5 McFarland standards and confirmed by measuring at 580 nm in a spectrophotometer. Final dilution of the cell suspensions in broth (1:100) gave approximate inoculum of 10^4 $CFUmL^{-1}$ compared with McFarland standard.

Susceptibility Assays

Antibacterial activities were evaluated using agar diffusion and micro dilution methods against the selected bacteria.

Susceptibility was determined by agar well diffusion technique. Agar plates were spread with 100μL of 0.5 McFarland solutions of the bacterial strain cultures in 0.85% Sterile Distilled Water (SDW). A cooled flamed cork borer (5 mm) was used to bore four wells in each plate and the agar plugs were removed using a sterile needle. 50 µL of the positive control drug Ciprofloxacin, (0.0125 mg/mL) and 50 mg/mL of nutrient broth (negative control) were added into the first and second wells, while into the remaining wells, 50 mg/mL of the essential oils dissolved in DMSO were added respectively, the culture plates were then incubated for 24 h at 37°C. The diameter of clear zone around each well, indicating the activity of the plant extract against the organisms, was measured in mm.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentrations (MIC) of the essential oils were determined using 96-well microplates by the broth micro dilution method (Otang *et al*., 2012). Sterile distilled water (120 µL) was added into each well of the first (A) and last (H) rows as well as wells of the last column. Then, 120 µL of Nutrient Broth (NB) was added into each well of the second row (B). 150 µL of NB was added into the remaining wells of the first column, 100 µL into the rest of the wells from the second column rightward; fifty microliters of the essential oils was then added into the third well of the first column while 50 µL of the positive (Amoxicillin) and negative control (SDW) were separately added into the remaining wells of the first column. Serial dilutions of the samples (essential oils or antibiotics) gave concentrations ranging from 0.005 to 5 mg/mL. Subsequently, 20 µL of 0.5 bacterial suspensions was inoculated into the wells except those which contained SDW. Growth of bacteria before and after incubation was measured at 620 nm with an automatic microplate reader (SynergyMx BiotekR, USA). The lowest concentration of the test agent at which 50% bacterial growth was inhibited is the MIC. The differences between the absorbances of test wells and control wells that contained the broth and antimicrobial agent alone without the test bacteria was calculated as the growth rate.

In-Vitro Anti-Inflammatory Activity

Protein Denaturation

Bovine Serum Albumin (BSA) was used to determine the inhibition of protein denaturation (Murugan and Parimelazhagan, 2014). To the reaction mixture made up of 0.45 mL bovine serum albumin (5% w/v, pH 6.3) and 0.05 mL of distilled water, was added 1000 µg of the essential oils from *M. oleifera* parts (mg/ml of DMSO), incubated for 30 min at 37°C and then heated at 57°C for 5 min. The samples were cooled and 2.5 mL of phosphate buffer solution was added and the absorbance measured at 600 nm. For negative control, 0.05 mL distilled water and 0.45 mL of bovine serum albumin was used, while Diclofenac was used as the reference drug. Inhibition of protein denaturation was calculated as:

$$\text{Inhibition of protein denaturation (\%)} = [\text{Abs control- Abs Sample/Abs Control}] \times 100$$

Membrane Stabilization Assay

Hypotonic solution-induced rat erythrocyte haemolysis was used to assess the membrane stabilizing activity of the essential oils. Rat erythrocyte cells were prepared as described previously (Oyedapo *et al*., 2010). Briefly, whole blood was obtained with heparinized syringes from a rat through cardiac puncture. The blood was washed three times with isotonic buffered saline solution at pH 7.4 and centrifuged each time for 10 min at13000 rpm to obtain the stock erythrocytes (red blood cells). For the membrane stabilizing assay, the test sample consisted of essential oil or standard drug at concentrations ranging from 0.1 to 0.5 mg/mL, plus stock erythrocyte suspension (0.5 mL, mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10mM sodium phosphate buffered saline (pH 7.4). The control consisted of 0.5 mL of RBC mixed with hypotonic-buffered saline solution alone. The mixtures were incubated for 10 min at room temperature, centrifuged at 13000 rpm for 10 min and absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated as:

$$\text{\% Inhibition of haemolysis} = 100 \times [\text{OD1-OD2/OD1}]$$

where, OD1 is the optical density of hypotonic-buffered saline solution alone and OD2 is the optical density of test sample in hypotonic solution. Diclofenac was used as the standard drug.

Statistical Analysis

The data were expressed as mean values ± standard deviation of three replicates and were subjected to Analysis of Variance (ANOVA). Means were considered significant at $p<0.05$. All analyses were done using MINITAB student version 12 for Windows software.

Results

Chemical Composition

The essential oils were obtained separately by solvent free microwave extraction method from the dried

flowers, leaves, seeds, barks and roots of *Moringa oleifera.* The oil yields ranged from 0.3% in the seed to 3.6% in the leaves (Fig. 1). The composition and retention indices of the essential oils from different parts of *Moringa oleifera* are presented in Table 1.

A total of 47 compounds, making up 90.35%, 78.99%, 92.77%, 96.59% and 99.99% from the flowers, leaves, seeds, barks and roots respectively were identified. Eicosane was the major component (20.93%, 17.12% and 21.59%) of the oils from the flowers, leaves and seeds respectively, while the oils from the barks and roots had naphthalene (18.40%) and benzene isothiocyanato methyl (35.83%) as the main components.

Table 1: Chemical composition (%) of essential oils extracted from the different plant parts of *M. oleifera*

Compound	RI	Flowers	Leaves	Seeds	Barks	Roots
Trans-2,7-dimethyl-3,6-octadien-2-ol	710	-	0.68	-	3.60	-
Benzaldehyde	737	-	-	0.66	7.14	13.43
α-thujone	834	-	-	-	2.86	-
Eucalyptol	949	0.58	1.30	0.87	8.06	-
Nonanal	966	3.5	1.89	2.24	10.36	-
Benzyl nitrile	993	-	-	-	4.65	14.17
2-Bornanone (Camphor)	996	3.94	2.65	3.97	10.65	-
Benzene acetaldehyde	1002	0.49	1.27	0.61	5.72	-
2-Isopropoxyphenol	1005	-	-	2.49	9.68	-
Terpinen-4-ol	1007	0.42	-	-	-	-
Naphthalene	1011	-	-	13.41	18.40	-
α-Terpineol	1012	0.36	0.37	-	-	-
Estragole	1013	-	-	1.03	-	-
Anethole	1022	-	-	1.51	5.95	-
Benzene, isothiocyanato methyl	1046	-	-	-	-	35.83
Methyl eugenol	1086	0.66	1.14	2.72	-	1.04
Heptadecane	1092	1.56	1.04	1.37	-	-
2-Pentadecanone, 6,10,14-trimethyl	1100	1.65	1.00	3.02	-	0.90
Megastigmatrienone	1101	0.56	-	-	-	-
Nonadecane	1105	2.98	3.37	2.30	-	-
Phthallic acid, isobutylundecyl ester	1109	1.78	1.51	6.38	-	2.71
Nerolidol	1111	12.55	0.72	8.79	-	-
Trans-beta-Ionone	1117	0.89	1.23	1.43	-	-
Heptadecanal	1123	-	0.83	-	-	-
Isopropyl myristate	1124	-	-	-	-	1.01
α-Cardinol	1135	-	1.30	-	-	-
Octadecane	1138	-	3.55	3.46	-	0.63
Hexadecanoic acid, methyl ester	1149	1.1	-	-	-	-
Benzophenone	1154	-	-	2.00	-	-
Spathulenol	1157	-	1.19	-	-	-
Curan-17-oic acid, 2,16-didehydro-20-hydroxy-19-oxo-, methyl ester	1159	-	1.41	-	-	-
Methyl salicylate	1173	-	-	-	5.62	-
n-Hexadecanoic acid	1185	5.90	7.33	-	-	-
Phenanthrene	1186	-	-	1.25	-	-
Dibutyl phthalate	1188	1.51	5.19	5.19		24.95
Hexadecanal	1198	3.93	-	-	-	-
Phytol	1203	0.81	2.34	-	-	-
Pentadecanal	1204	2.71	4.10	2.04	-	-
Benzaldehyde, 4-methoxy	1223	-	-	-	3.90	-
Bicyclo[10.8.0] eicosane, cis-	1226	4.36	3.43	-	-	-
1-Naphthalenamine, N-phenyl-	1233	1.29	1.76	4.44	-	-
Eicosane	1282	20.93	17.12	21.59	-	0.73
Heptacosane	1919	12.04	9.13	-	-	-
1,22-Docosanediol	2246	3.85	-	-	-	-
1-Ascorbic acid 2,6-dihexadecanoate	2299	-	1.12	-	-	2.97
Total		90.35	78.99	92.77	96.59	99.99

Note: RI- Retention Indices

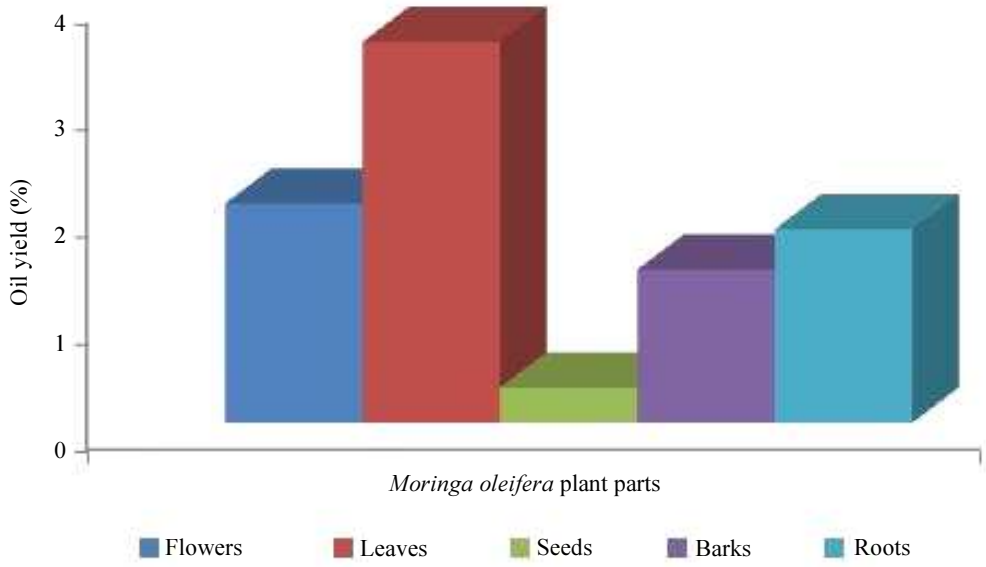

Fig. 1: Essential oil yields from the different plant parts of *Moringa oleifera* *Values are means ± SD (n = 3). Bars with different superscripts are significantly different (p<0.005)

Table 2: Inhibition zone diameter (mm) of essential oils from the different plant parts of *M. oleifera*

Bacterial strains	Flowers	Leaves	Seeds	Barks	Roots	Ciprofloxacin
Gram positive						
Enterococcus faecalis	16±1.41[a]	16±2.83[a]	18±1.41[b]	14±0.71[c]	16±2.12[a]	44±2.83[d]
Gram negative						
Klebsiella pneumoniae	19±4.24[a]	25±2.83[b]	17.4±0.71[c]	21±9.19[d]	15±2.12[e]	46±2.12[f]
Proteus vulgaris	15±2.12[a]	21±2.83[b]	21±4.95[b]	19±2.12[c]	17±1.41[d]	47±1.41[e]
Shigella flexineri	20±0.00[a]	19±7.07[a]	20±7.07[a]	18±0.00[c]	23±4.24[d]	53±6.36[e]

[*]Values are means ± SD; n = 3; Means with different superscripts in the same row are significantly different (P<0.05). Ciprofloxacin, reference antibiotic

Table 3: Minimum Inhibitory Concentration (MIC) of essential oils from the different plant parts of *M. oleifera*

Bacterial strain	Flower	Leaf	Seed	Bark	Root	Ciprofloxacin
Enterococcus faecalis	>5	>5	5	5	>5	>5
Shigella flexineri	>5	>5	>5	>5	>5	>5
Proteus vulgaris	1.25	>5	5	>5	>5	5
Klebsiella pneumoniae	2.5	2.5	1.25	>5	>5	2.5

Note: MIC, Minimum Inhibitory Concentration in mg/mL

Antibacterial Activity of the Essential Oils

The result of the agar well diffusion assay and the MICs of the extracts are summarized in Tables 2 and 3. Of the 8 bacterial isolates tested, *3 gram-ve (Klebsiella pneumonia, Proteus vulgaris and Shigella flexineri)* and only 1 Gram +ve (*Enterococcus faecalis)* exhibited susceptibility to the oils with inhibition zones ranging from 14.00 to 25.00 mm. All the other bacterial isolates (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* ATCC 19582 and *Serratia marcescens* ATCC 9986) showed no susceptibility to any of the essential oils. The highest antibacterial activity was observed with the leaf essential oil (25 mm) against *K. pneumonia* and the root oil (23 mm) against *S. flexinerii* respectively.

Minimum inhibitory concentration (MIC_{50}) for the oils (Table 3) ranged from 1.25 mg/mL for the seed oil to >5 mg/mL in bark for *K. pneumonia*; while for *P. vulgaris* it was 1.75 mg/ml for the flower and 10 mg/ml for the leaf essential oils respectively. For both *S. flexineri* and *E. faecalis*, MIC_{50} was >5 mg/mL, while for the reference drug Ciprofloxacin, MIC_{50} was >5 mg/mL for all the bacterial strains tested except for *K. pneumoniae* which was 2.5 mg/mL.

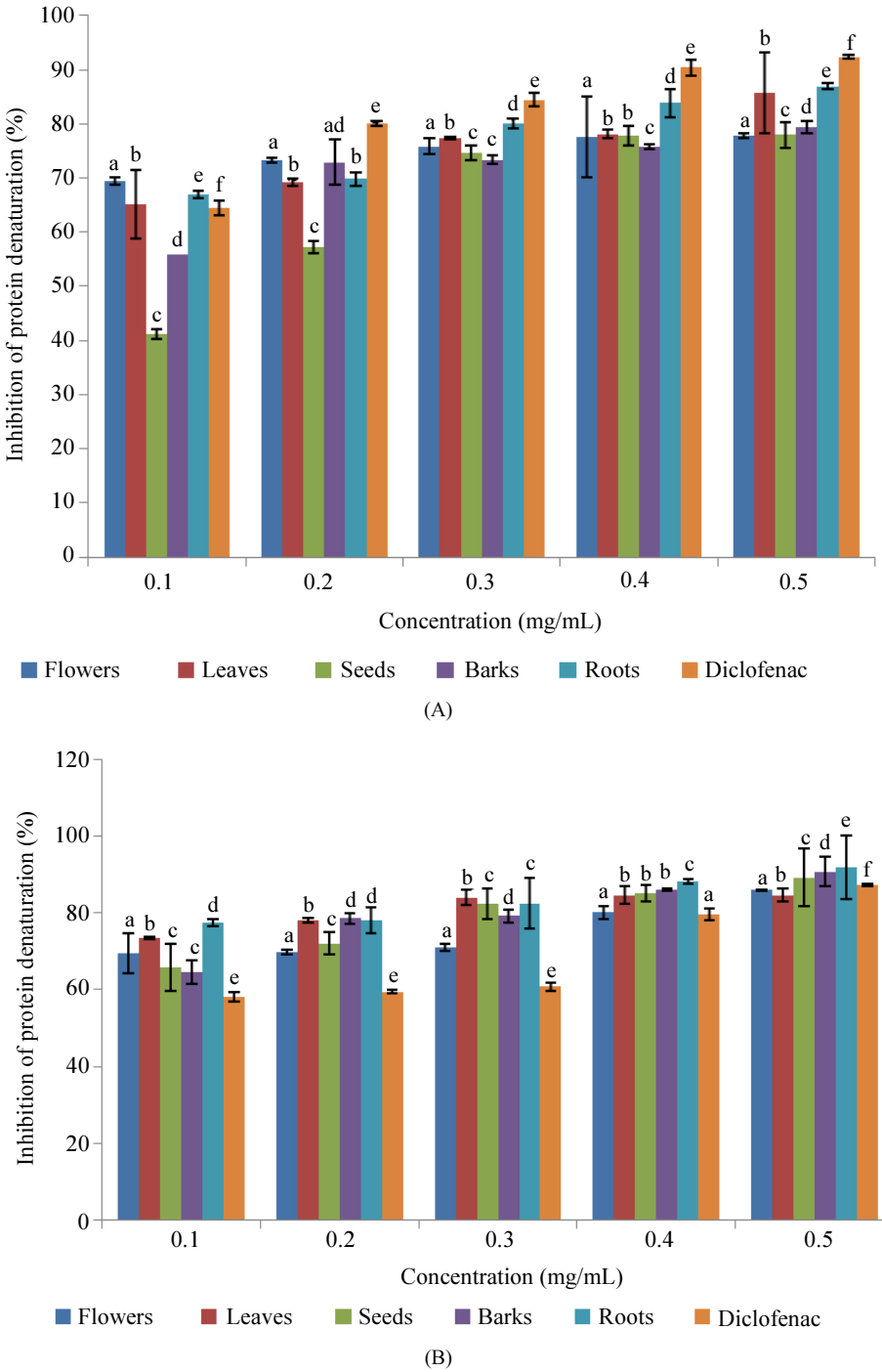

Fig. 2: Activity of essential oils extracted from the various parts of *M. oleifera* on (A) inhibition of heat induced protein denaturation and (B) erythrocyte membrane stability; *Values are means ± SD (n = 3). Bars within the same concentration with different superscripts are significantly different ($p<0.005$)

Anti-Inflammatory Activity

Essential oils from the different parts of *M. oleifera* showed very high ($p<0.05$) concentration-dependent inhibitory activity against protein (albumin) denaturation (Fig. 2A). All the oils, exhibited inhibition against protein denaturation at $IC_{50} < 0.1$ mg/mL, except the leaf oil which showed a higher IC_{50} at 0.2 mg/mL. The membrane stabilizing action of the essential oils followed the same trend as observed for protein

denaturation (Fig. 2B). Erythrocyte membrane stability was enhanced in a concentration-dependent manner, with oil from the roots exhibiting the highest inhibition (77.50 %) of membrane haemolysis at IC_{50}: 0.1 mg/mL.

Discussion

The essential oils obtained from the different parts of M. oleifera showed variety in their composition. For instance, eucalyptol, benzene acetaldehyde, nonanal and camphor occurred in the flower, leaf, seed and bark essential oils but were absent in the root oil; while methyl eugenol, trans-beta-ionone and 1-naphthalenamine, N-phenyl were present only in the flower, leaf and seed. 1-(+)- Ascorbic acid 2,6-dihexadecanoate was present only in the leaf and root oils; anethole in seed and bark oils; nerolidol in flower, leaf and seed oils and phytol in flower and leaf oils alone. Only the essential oil from the bark contained Methyl salicylate while isopropyl myristate was found in the root oil alone. Interestingly, 1-(+) - Ascorbic acid 2, 6-dihexadecanoate was found to be higher (2.97%) in the root than in the leaf (1.12%).

Pino (2013) reported that the volatile fraction of Moringa flower essential oils was characterized by aliphatic compounds, nitrogen-compounds, oxygenated monoterpenes, monoterpene hydrocarbons and oxygenated sesquiterpenes. The presence of benzaldehyde, ionone, phytol, benzene acetaldehyde, hexacosane, pentacosane and heptacosane have also been reported (Chuang *et al*., 2007; Marrufo *et al*., 2013). According to Hussein *et al*. (2014), the major constituents of Moringa seed oil were naphthalene and benzene isothiocyanato methyl, while the main constituents of root oil were hydrocarbons, acids, esters, alcohols, aromatics and alkamides along with benzyl glucosinolate (Goyal *et al*., 2007; Faizi *et al*., 2014). This is similar to our findings and we also report that the main components of *M. oleifera* bark essential oil were naphthalene, nonanal, camphor and methyl salicylate.

All the different parts of *M. oleifera* essential oils showed strong broad spectrum activity against both Gram-positive and Gram-negative bacterial strains as shown by the zone inhibition diameters which were greater than 13 mm (Vieira *et al*., 2010); and the MICs. The various bioactive components of the essential oils could account for the differences observed in their antimicrobial effects.

Broad spectrum activity attributed to bactericidal substances or toxins have been reported earlier for water and ethanol-based moringa seed extracts (Vieira *et al*., 2010). According to Silva *et al*. (2011), the antimicrobial actions of essential oils are attributed mainly to phenolic compounds. The reports by Dorman and Deans (2000) that terpenes induced antimicrobial properties are corroborated by the results obtained in this study. However, other minor components such as limonene, linalool, γ-terpinene, p-cimene, α-pinene and α-terpineol could be responsible for antimicrobial activity due to synergistic effects (Van Vuuren and Viljoen, 2007). This is in agreement with our findings in this study as other components such as phenols, alpha-thujone and terpinen-4-ol were present in the essential oils.

Protein denaturation is one of the main causes of inflammatory and arthritic diseases (Chatterjee *et al*., 2012). In this study, inhibitory actions of the essential oils on denaturation of BSA and rat erythrocyte membrane haemolysis were used to evaluate anti-inflammatory activity. Agents that could prevent protein condensation, precipitation of denatured protein aggregates and inhibit the COX enzyme or protect the lysosomal membrane from breakdown are useful in attenuating inflammation and its attendant diseases (Rauf *et al*., 2014; Osman *et al*., 2016).

Essential oils from the various parts of *M. oleifera* protected the erythrocyte membrane against lysis induced by hypotonic solution in the order root oil>leaf oil>flower oil>seed oil>bark oil>Diclofenac. The ability of the essential oils to maintain erythrocyte membrane integrity was comparable and even higher than that of diclofenac the standard drug.

Because of the similarities between the erythrocyte and lysosomal membranes, prevention of the rupture of the erythrocyte membrane is an indication that such agents will protect the tissue against damage caused by the release of hydrolytic enzymes thus alleviating some symptoms of inflammation (Olajide *et al*., 2000; Tatiya and Saluja, 2011).

Research has shown that the main mechanisms of action of common anti-inflammatory drugs like aspirin and diclofenac is by preventing protein denaturation, enhancing membrane stability and inhibitory effect on cyclooxygenase (Umapathy *et al*., 2010; Odeyemi *et al*., 2015). The observed anti-inflammatory properties of *M. oleifera* essential oils could therefore be attributed to their capacity to inhibit thermal and hypotonic protein denaturation and enhance membrane stability. This is in agreement with reports on the aqueous, ethanol, chloroform and hydro-ethanol extracts of *M. oleifera* flower extracts (Alhakmani *et al*., 2013), leaves (Rao *et al*., 1999) and seeds (Minaiyan *et al*., 2014) on inflammatory models. For the first time, this study reports the anti-inflammatory effects of essential oils from *M. oleifera* parts. The observed anti-inflammatory activity could be attributed to benzene isocyanato methyl or isopropyl myristate in the root oil, methyl salicylate and alpha-thujone in the bark as well as other phytochemicals present in the various parts which can interfere with the early phase of inflammation to stop the cascade reactions (Matsuda *et al*., 2007; Vijayalakshmi *et al*., 2011). Although the mode of action is yet to be determined, it could be that the oils acted by inhibiting protein denaturation.

Previous studies have reported organ-dependant variations and distribution of essential oil components in plants such as *Conyza bonariensis* L (Mabrou *et al*., 2011), *Helichrysum armenium* (Oji and Shafaghat, 2012), *Ligusticopsis wallichiana* (Padalia *et al*., 2012), *Canarium parvum* Leen. and *Canarium tramdenanum* Dai et Yakovl. (Burseracea) (Thang *et al*., 2014).

The results agree with reports from previous studies; for instance, Methyl Salicylate (MeSa) is an important topical analgesic and anti-inflammatory agent which acts as a counter-irritant. MeSa is the active ingredient in topical liniments and ointments used in the management of rheumatic/ arthritic conditions (Paudel *et al*., 2013), as a flavouring agent in foods, beverages and confectionaries (Anderson, 2003) and as an insecticidal agent (Bossou *et al*., 2013). Benzene, Isothiocyanato methyl (BITC), the major component of oil from Moringa root, has been established as a therapeutic agent for treating leukaemia and pancreatic cancers (Batra *et al*., 2010). BITC acts as an antioxidant, antiproliferative and apoptotic agent that inhibits NF-κB binding to DNA; suppresses lipid accumulation and macrophage infiltration in tumor tissues. Previous studies have also established that BITC blocks important signaling pathways relevant to tumor progression and invasion (Rao, 2013). Megastigmatrienone, an aromatic flavor compound which produces some of the spice notes associated with certain wines is limited to the flower essential oil. Similarly, isopropyl myristate which is a good emollient used in cosmetic and medicinal preparation as a topical anti-fungal, anti-bacterial, anti-lice and anti-fleas was limited to the root oil alone. Alpha-Thujone present in *M. oleifera* bark oil has anthelmintic, anti-nociceptive and insecticidal actions (Hold *et al*., 2000). Bueno *et al*. (2011) reported that alpha cardinol; a sesquiterpene present in *M. oleifera* leaf oil, has anti-fungal, hepatoprotective properties and is a possible remedy for drug-resistant tuberculosis. The synergistic effects of these bioactive compounds in the essential oils could account for the antibacterial, anti-inflammatory and other folkloric therapeutic effects attributed to the whole plant. However, there is the need for caution in the use of the roots, bark and seed oils of *M. oleifera* because of the high content of phthalate compounds.

Conclusion

The study reports for the first time the comparative chemical composition, antibacterial and anti-inflammatory effects of essential oils from the flowers, leaves, seeds, barks and roots of *Moringa oleifera*. The presence of bioactive compounds such as benzene, isocyanato methyl and isopropyl myristate in the root oil, methyl salicylate and alpha-thujone and other phytochemicals are evidence of their potential therapeutic effects and the diverse nutritional and pharmacological uses of *M. oleifera* traditionally. The possibility of *M. oleifera* root oil as a lead for anti-cancer drug could be further explored considering the high concentration of benzene isothiocyanato methyl.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author's Contributions

Gloria Aderonke Otunola: Conceptualized and designed the study, performed the experiments. Contributed equally to the writing, proof reading and approval of the final version of this manuscript.

Anthony Jide Afolayan: Participated in the concept and design of the study. Contributed equally to the writing, proof reading and approval of the final version of this manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that both authors have read and approved the manuscript and there are no ethical issues involved.

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