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Article · March 2017

DOI: 10.9734/EJMP/2017/31834

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European Journal of Medicinal Plants
18(1): 1-9, 2017; Article no.EJMP.31834
ISSN: 2231-0894, NLM ID: 101583475

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Chemical Composition and *In-vitro* Antibacterial Activity of the Essential Oil of Nigerian *Moringa oleifera* Lam. Flowers

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Authors' contributions

This work was carried out in collaboration between all authors. Author OSB designed the study and wrote the first draft of the manuscript. Author RYF managed the literature searches. Authors OAF, DAA and CAO managed the analyses of the study and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2017/31834
Editor(s):
(1) Ghalem Bachir Raho, Biology department, Sidi Bel Abbes University, Algeria.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Daniela Hanganu, Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Romania.
(2) Wan Mohd Nuzul Hakimi Wan Salleh, Universiti Teknologi Malaysia, Malaysia.
(3) Karolina Ratajczak, The Poznan University of Life Sciences, Poland.
Complete Peer review History: http://www.sciencedomain.org/review-history/18012

Original Research Article

Received 27th January 2017
Accepted 23rd February 2017
Published 2nd March 2017

ABSTRACT

Aims: To investigate the chemical composition and antibacterial activity of essential oil isolated from flowers of *Moringa oleifera* (*MO*) grown in Western Nigeria.
Methodology: Gas chromatography–mass spectrometry (GC-MS) analysis was carried out for identification and determination of the phytochemical constituents of the oil. Standard microbiological methods was also employed to evaluate the antibacterial activities of the oil.
Results: GC-MS analysis revealed a total of twenty-five phytochemical constituents, with the major constituents found to be nonanal (17.3%), trans-geranyl geraniol (13.5%) and eicosane (12.3%), α-terpineol (7.2%), methyl palmitate (4.6%) and methyl octadec-9-enoate (4.1%). The antibacterial assay, using standard microbiological methods, showed that the oil had inhibitory effects against

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both Gram-positive (*Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus)* and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria isolates. The minimum inhibitory concentrations (MICs) exhibited by the essential oil against test bacteria ranged between 1.25 mg/mL and > 5 mg/mL.

Conclusion: Essential oil of Nigerian *Moringa oleifera* flowers contains biologically active ingredients and possess some level of antibacterial activity. The oil can be a good source of antibacterial agents.

Keywords: Moringa oleifera; essential oil; chemical composition; flowers; nonanal; geranyl geraniol; antibacterial activity.

1. INTRODUCTION

Different plant parts (bark, roots, fruits/seeds, leaves, stem and flowers) contain volatile oils, usually referred to as essential oils and the study of their chemical composition has revealed that these oils consist primarily of highly functionalized chemical classes of organic compounds, including monoterpenoids, sesquiterpenoids, etc. [1].

The major roles of essential oils in nature centers on plant defense against microorganisms, animals feeding on plants (herbivores) and insects, attraction of insect pollinators and fruit-dispersing animals, water regulation and plant-plant (allelopathic) interactions [2,3]. Also, they are valuable natural raw materials for the agronomic, cosmetic, food, perfume, pharmaceutical and sanitary industries [4].

Over hundreds of years, bacteria have developed antibiotic resistance mechanisms, hence they have become resistant to most of the developed natural antimicrobial agents. This resistance has led to increasingly limited effectiveness of current antimicrobial drugs. Therefore, new antimicrobial agents that are active against resistant bacteria are required [5]. A wide variety of essential (volatile) oils from aromatic and medicinal plants have been shown to exhibit biological activity, including antibacterial, antifungal, and antioxidant properties [3].

Moringa oleifera Lam, which is indigenous to south Asia, is the most widely cultivated species of a monogeneric family, the Moringaceae [6]. It ranges in height from 5 to 10 m and it has been introduced and become naturalized in other parts of the world, including the Arabian peninsula, Southeast Asia, West Asia, the Pacific and Caribbean Islands, South and North America, East and West Africa [7]. Different parts of the plant (bark, flowers, fruit, leaves, root, seeds and immature pods) have been reported to possess impressive medicinal uses and pharmacological properties, including antibacterial, diuretic, anti-carcinogenic, antifungal, anti-inflammatory, anti-nociceptive antioxidant, anti-sickling, antispasmodic and antiulcer properties and high nutritional value [8-13].

In Nigeria, the decoction of the leaves is commonly used in the treatment of fevers (typhoid and malaria), sore throat, bronchitis and diabetics [14]. Other parts such as stem, root and flower found their folkloric applications in the treatment of various ailments like rheumatism, inflammations, articular pains, aphrodisiac, kidney pain and constipation.

Moringa leaves have been shown to be rich in vitamins and minerals. For example, the fresh leaves have been reported to contain vitamins A and C more than those reported in carrots and oranges [15], more iron than spinach and as a rich source of other nutritive phytochemicals, minerals and antioxidants such as ascorbic acid, flavonoids, phenolics, carotenoids, calcium and potassium [16]. Other classes of compounds that have been isolated from the plant include glucosinolates and isothiocyanates from the seeds and leaves, which have contributed to its hypotensive, anticancer, and antibacterial activity [17,18]. Also isolated from the bark are 4-hydroxymellin, moringin, moringinine, β-sitosterol, β-sitostenone (Stigmast-4-en-3-one), octacosanoic acid and vanillin [10,19], some of which have been suspected to mediate the hypoglycemic effect of the plant [8] and anti-proliferative effect on human tumour lines (HepG2 and MCF-7) [20]. Other phytochemical constituents of extracted oil of the plant seeds have been reported [21-22]. However, little information could be found in the literature about the composition of the essential oils of the different parts of *M. oleifera* and their biological properties [15,23,24a,b].

To the best of our knowledge, a detailed investigation of the volatile oil of *M. oleifera* flowers grown in Nigeria has not yet been undertaken. Also, the antibacterial effect of *M. oleifera* flower oils has not been reported to date. Therefore, the aim of the present work is to determine the chemical profile of the essential oil from the flowers of *M. oleifera* grown in Obafemi Awolowo University, Nigeria and describe its antibacterial activity.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Moringa oleifera flowers were collected from plants growing at Obafemi Awolowo University, Ile-Ife, Nigeria in November 2013. It was identified and authenticated at the herbarium unit of Department of Botany, of the same institution. A voucher specimen (no.IFE-17606) for *Moringa oleifera* was deposited in the herbarium.

2.2 Extract Preparation of the Plant Material

The air dried flowers (265 g) were pulverised and subjected to hydrodistillation using a Clevenger-type apparatus for 3 hours after which the moisture content of the oil was removed through the use of anhydrous sodium sulphate. The yields (w/w %) were estimated based on the weight of the plant material before distillation. The essential oil was kept in an air-tight dark vial and refrigerated at 4℃.

2.3 Chemicals and Reagents

Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific (Leicestershire, UK). Nutrient agar medium (Mast Group, Bootle, UK) was used for sub-culturing the test organisms, while Mueller-Hinton agar medium (Mast Group, Bootle, UK) was used for the sensitivity testing. All reagents were analytical standard.

2.4 Gas Chromatography-mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of the oil was performed using Agilent GC/MS (5915/1890N) with an HP-5ms fused silica capillary with a (5% phenyl)-poly-methylsiloxane) stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The GC oven initial temperature was 50℃ and gradually increased to 100℃ at rate of 5℃ /min and finally to 280℃ at the rate of 10℃ / min. Helium gas at flow rate of 1 mL/min was used as carrier gas and the sample was injected in split mode (50.2:1). The GC was coupled to Mass Selective Detector Transfer Line Heater maintained at 270℃. Identification of compounds was based on comparisons of the relative retention time and mass spectra with those of the Wiley Registry of Mass Spectral Data (John Wiley & Sons, Inc./ Hoboken, NJ, USA) and NIST/EPA/NIH Mass Spectral Library (National Institute of Standards and Technology/Gaithersburg, MD, USA) of the GC-MS. The percentage composition was computed from the peak areas of the GC spectra.

2.5 Bacterial Strains

Antibacterial activity was assessed against the bacterial isolates comprising of National Collection for Industrial Bacteria (NCIB), collected from Culture Collection of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. Bacterial isolates used for this study include typed cultures: *Staphylococcus aureus* (NCIB 8588), *Micrococcus luteus* (NCIB 196), *Bacillus cereus* (NCIB 6349), *Escherichia coli* (NCIB 86) and *Pseudomonas aeruginosa* (NCIB 950).

2.6 Antibacterial Sensitivity Testing

The antibacterial sensitivity screening of the essential oil was determined in accordance with agar-well diffusion method described by Russell and Furr [25] and Irobi et al. [26] with little modification. The bacterial isolates were sub-cultured into nutrient broth before use. The 18-hour-old bacterial culture was standardized using McFarland standard (10^6 cfu/mL of 0.5 McFarland standard). One hundred microliters of each of the standardized bacterial suspension was evenly spread on Mueller-Hinton agar medium using a sterile glass spreader. Sterile cork borer was used to bore holes into the agar medium allowing about 5 mm distance to the edge of the plate. The plates with bacteria cultures were treated with the solution of the oil at a final concentration of 25 mg/mL. The plates were allowed to stand on the laboratory bench for one hour to allow for proper diffusion of the oil solution into the medium. The plates were incubated at 37℃ for 24 hours after the plates were observed for zones of inhibition. The effect of the oil on bacteria was compared with that of streptomycin at a concentration of 1 mg/mL.

2.7 The Minimum Inhibitory Concentrations (MICs) of the Oil against the Test Organisms

The method described by Akinpelu and Kolawole [27] was used to determine the MIC of the oil against the test isolates. A two-fold dilution of the oil was prepared and 2 mL aliquots of different concentrations of the solution were added to 18 mL of pre-sterilized molten nutrient agar medium 40℃. The medium was poured into sterile Petri dishes and allowed to set. The plates were left on the laboratory bench for 24 hours to observe their sterility. The dry surface of the media was later streaked with standardized 18-hour-old bacteria culture. The plates inoculated with bacterial culture were incubated at 37℃ for up to 72 hours. These were later examined for the presence or absence of growth. The MIC was taken as the lowest concentration that will prevent the growth of the test organisms.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of the Oil

A colourless oil of 0.74 g (0.28% w/w) was obtained by hydrodistillation. The GC-MS analysis led to the detection of 29 compounds of which 25 were identified and quantified, which made up 89.9% of the oil. The GC-MS chromatogram of the volatile constituents of the oil is shown in Fig. 1, while the chemical constituents are listed in Table 1, in order of elution from an HP-5MS capillary column. The identified compounds were distributed into ten classes of compounds as shown on Table 2. Aldehydes were the most abundant with percentage composition of 19.4%, followed by hydrocarbons (aromatic and aliphatic), monoterpenoids, diterpenoids and esters with percentage compositions of 14.7%, 13.9%, 13.5% and 11.8% respectively (Table 2). Previous work on the essential oil from the leaves of the plant showed the monoterpenoids as the most abundant class of compounds (81.8%) [24a]. Of the 25 compounds identified in the oil of the flowers (Table 1), nonanal (17.3%), trans-geranyl geraniol (13.5%) and eicosane (12.3%) were the predominant compounds, compared to α-phellandrene (25.20%) and p-cymene (24.9%) found in the essential oil of the leaves of *M. oleifera* from Nigeria [24]. Also, the GC-MS analysis of the essential oil of *M. oleifera* leaves from Taiwan indicated preponderance of pentacosane (17.41%) and hexacosane (11.20%) [23].

The principal constituents identified in the essential oil were compounds of pharmacological interest. Although saturated aldehydes (including nonanal which is the most abundant constituent (17.3%) in the studied oil) extracted from olive flavor did not exhibit significant antibacterial activity [28], nonanal, has been reported to show a significant symptomatic relief on mice with induced diarrhoea [29] and completely inhibited mycelial growth or sclerotia formation, thus suggesting its potential role in biological control [30].

The diterpene alcohol, geranylgeraniol which was present in a relatively considerable amount (13.5%) is reputed for broad spectrum of biological activities, as a potentially useful chemopreventive agent in hepatocarcinogenesis [31] and apoptosis of carcinogen cells [32,33], potent and selective inhibitors of M. tuberculosis [34] and therapeutic action against Chagas disease [35]. Monoterpenoids such as ɣ-terpinene (3.4%) and α-terpineol (7.2%) present in the oil have also been reported to demonstrate good anti-acetylcholinesterate and insecticidal activities [36,37]. In addition, α-terpineol, has been reported to increase the permeability of skin to lipid soluble compounds, exhibits anticancer, anti-inflammatory and antimicrobial properties and anti-proliferative effects on human erythroleukaemic cells [38-40].

3.2 Antibacterial Assay

The results of the susceptibility screening test of the essential oil are presented in Table 3. Several studies have reported the antimicrobial property of essential oils in which the activity was attributed to the complex interaction between (or combinatory effects of) the different classes of compounds such as hydrocarbons, alcohols, aldehydes and ketones, esters, ethers, organic acids or phenols found in the oils [3,41-43]. In addition, it has been reported that essential oils consisting of only terpene hydrocarbons exhibit little or no antibacterial activity, while those containing terpene alcohols, ketones or esters (such as farnesol, terpineol, α-thujone, geranyl acetate, etc) showed moderate to good activity and those containing α, β-unsaturated aldehydes or phenolics, such as cinnamaldehyde, citral, carvacrol, eugenol, etc, showed the highest antibacterial activity [44-46].

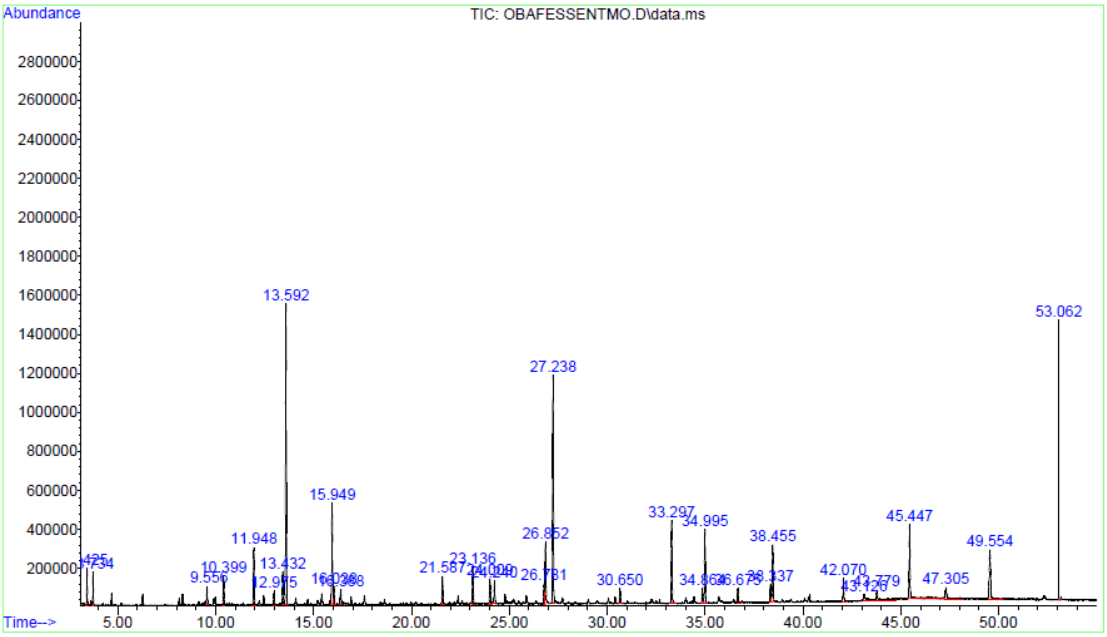

Fig. 1. GC-MS chromatogram of the volatile constituents of *Moringa oleifera* Lam. flowers

Table 1. Chemical composition of essential oil from the flowers of *M. oleifera*

Compounds	RI	*RI (Literature)	% Composition
n-Butyl acetate	812	809	1.3
2-Hexenal	853	855	0.6
Heptanal	902	902	0.7
Benzaldehyde	960	957	0.8
2-Pentyl furan	991	994	1.1
4-Carene	1001	1001	2.6
ɣ-Terpinene	1059	1058	3.4
1-Octanol	1071	1073	0.6
Nonanal	1103	1103	17.3
Naphthalene	1180	1183	1.1
α-Terpineol	1189	1190	7.2
Dodecane	1200	1200	1.3
Isogeraniol	1235	1236	0.7
Caryophyllene	1406	1406	2.5
Geranyl acetone	1450	1452	1.8
Nerolidol	1540	1541	3.8
Methyl tridecanoate	1626	1626	0.9
Hexahydro farnesyl acetone	1845	1842	5.1
Methyl palmitate	1926	1927	4.6
Eicosane	2000	2000	12.3
hexadecyl acetate	2010	2012	0.9
Geranyl geraniol	2024	2024	13.5
methyl octadec-9-enoate	2110	2109	4.1
Squalene	2835	2835	1.1
Others			5.4
Total			**95.3**

** Adams [48]; Goodner [49]; Babushok et al. [50]*

Table 2. Class of compounds from essential oil of *M. oleifera* flower

Class of compound	% Composition
Hydrocarbons	14.7
Monoterpenoids	13.9
Diterpenoids	13.5
Sesquiterpenoids	6.9
Triterpenenoid	1.1
Acyclic alcohols	0.6
Aldehydes	19.4
Ketones	6.9
Esters	11.8
Heterocycle	1.1
Unidentified	5.4
Total	**95.3**

Table 3. Zones of inhibition (ZI) and minimum inhibitory concentrations (MICs) of the essential oil of *Moringa oleifera* flowers against the tested bacterial isolates

S/N	Organisms	Essential oil (25 mg/ml)		Streptomycin (1 mg/ml)	
		ZI (mm)**	MIC (mg/ml)	ZI (mm)**	MIC (mg/ml)
1	*Bacillus cereus*	18 ± 0.55	1.25	21 ± 0.57	0.0313
2	*Micrococcus luteus*	15 ± 0.55	4.05	18 ± 0.41	0.0313
3	*Staphylococcus aureus*	13 ± 0.40	3.25	20 ± 0.57	0.250
4	*Escherichia coli*	10 ± 0.33	> 5.0	11 ± 0.57	0.500
5	*Pseudomonas aeruginosa*	12 ± 0.60	>5.0	22 ± 0.66	0.250

*** = Mean of three separate experiments*

In this study, the essential oil was active against all the tested strains (three Gram positive {*Bacillus cereus, Micrococcus luteus* and *Staphylococcus aureus*} and two Gram negative bacteria {*Escherichia coli* and *Pseudomonas aeruginosa*}) at a concentration of 25 mg/mL. The zone of inhibition observed ranges between 10 ± 0.33 mm and 18 ± 0.55 mm, with the oil exhibiting more activity on Gram-positive bacteria. However, the standard antibiotic tested (streptomycin) showed higher inhibition than the essential oil against the 5 bacterial isolates at 1 mg/mL, with the zone of inhibition ranging between 11 ± 0.57 mm and 22 ± 0.66 mm. The degree of the antibacterial activities of essential oils has been attributed to the hydrophobicity of the components, with the Gram-negative bacteria considered less susceptible because they possess cell membrane restricting the diffusion of hydrophobic compounds through its lipopolysaccharide covering [47].

Antibacterial activity for the bacterial strains was also evaluated using minimum inhibitory concentration (MIC) (Table 3). The (MICs) results showed that the essential oil of *Moringa oleifera* flowers exhibited a relatively weak antibacterial activity against both Gram-positive and Gram-negative bacteria, but varied according to the type of bacterium, with a MIC ≥ 1.25 mg/mL, while the MIC for the standard antibiotic, streptomycin, varied between 0.031 mg/mL and 0.500 mg/mL. In the work of Marrufo et al. [24b], antibacterial screening of the essential oil of the leaves of *M. oleifera* showed that *Bacillus cereus* (a Gram positive bacterium) was the most sensitive strain, with an inhibition halo of 5.7 mm at just 2 µg/plate, while the Gram-negative bacterium (*Pseudomonas aeruginosa*) was inhibited by 5 µg/plate of essential oil. The MIC values were not determined.

To our knowledge, the present work is the first report to provide information on the antibacterial effect for the essential oil of flowers of *M. oleifera*.

4. CONCLUSION

The oil can be a good source of antibacterial agents, however in vivo studies and clinical trials would be required to assess the potential of the oil as an antibacterial agent in topical and/or oral applications.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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