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Research Article

Studies of the Antibacterial, Phytochemical, Proximate and GC-Mass Spectrophotometric of *Ocimum gratissimum* Linn Methanol Leaf Extract

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ABSTRACT

From decades, the effective treatment of diseases with folklore is basically with plants of medicinal values. Methanol leaf extract of *Ocimum gratissimum* was analysed for phytochemical constituents, nutritive value and chemical composition for possible antibacterial activity in comparison commercial antibiotics. The study confirms the presence of valuable plant chemicals for antimicrobial potency. Quantitatively, methanol extract of the vegetal showed high concentrations of 741.5, 346.95 and 137.5 mg/100g in saponin, flavonoids and alkaloids respectively. Nutritionally, it contained high percentage of 55.76, 18.18, 11.53 and 8.70 in carbohydrate, protein, moisture and crude fibre respectively. Highest inhibition recorded with the methanol leaf extract was 31 mm against *Corynebacterium accolens* while least inhibition of 14 mm was recorded against *Salmonella typhi*. The GC-MS composition of the essential oil extract showed the presence of Benzeaceticacid,.alpha.-[(diphenylmethylene)] (24.52%). The essential oil most inhibited Gram positive bacteria with highest zone of 33.20 mm and least inhibition of 24.00 mm while highest inhibition on Gram negative bacteria was 31.18 mm and least inhibition of 23.01 mm.

Key words: Bacteria inhibition, Proximate composition, GC, Phytochemicals

INTRODUCTION

Leafy vegetable species contain various medicinal and therapeutic agents which range from arrays of sedatives, wound healing, laxatives and sleep inducing components (Ajiboye *et al*., 2014). To a large extent, commonly used species of leafy vegetables have been studied in Nigeria (Kola, 2004). Several vegetable species thrive in Nigeria and other West African countries where they are used in human diet, as condiments or spices, as livestock feed supplement and as medicine (Ajiboye *et al*., 2014). The medicinal values of these vegetables lie in their bioactive constituents such as flavonoids, tannins, alkaloids, glycosides and phenolic compounds (Falodun *et al*., 2003). The information acquired from research on the bioactive constituents is generally important towards the discovery of new anti-infective agents from plants (Duraipandiyan *et al*., 2006).

Ocimum gratissimum Linn is a valuable leafy vegetable used since ancient times. It is also called African basil, "Efinrin" in Yoruba language of the South-western Nigeria (scent leaf) (Ajiboye *et al*., 2014). It belongs to the family *Lamiaceae* comprising more than 150 species and it is marked by its strong aroma and astringent taste (Sheelu *et al*., 2017). It grows widely throughout the temperate regions of the world (Sheelu *et al*., 2017). Different parts of the whole plant including the leaves, stems, flowers, roots and seeds are useful as it has a wide spectrum of pharmacological activities (Dzoyem *et al*., 2017). Documented reports shows that it possesses antifungal, antimalarial, antiviral, anaesthetic, antiprotozoal, anthelmintic agents, antidiabetic, anti-inflammatory, anti-stress and antibacterial activities (Oparacha *et al*., 2010; Cohen, 2014; Remia *et al*., 2017; Sheelu *et al*., 2017). Its essential oil has been reported to contain antifungal, insecticidal and nematicidal properties (Dubey *et al*., 2000; Remia *et al*., 2017).

The leaves and flowers of *O. gratissimum* are traditionally used as digestive, aromatic, galactogogue, tonic agents, for the treatment of diarrhoea, fever, ophthalmic skin diseases and upper respiratory tract infections and for insect bites (Sheelu *et al*., 2017). It is believed to promote longevity, serves as a decongestant for colds, bronchitis and sinusitis and the leaves when chewed after meals acts as a digestive agent. The seeds are edible and when soaked in water become mucilaginous which is

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a good pharmaceutical adjuvant, specifically a decaying agent (Ravikumar *et al.*, 2007; Dzoyem *et al*., 2017). It is believed that medicinal properties of this plant are as a result of the phytochemicals and essential oil present in the leaves, stem and roots. Secondary metabolites reportedly present in the leaves include Ursolic acid, Rutin, Eugenol, Apigenin, Salvigenin, Quercetin, Carnosic acid, Caffeic acid, Rosmarinic acid, Circhoric acid, Sitosterol and Transferulic acid (Grayer *et al*., 2000; Ouyang *et al*., 2013). Thymol, orintin and vicenin are also present in great amount. It also contains Terpenes, Lactone and Xanthenes (Sheelu *et al*., 2017).

Various studies carried out on this plant in Ado-Ekiti have been reported but with little studies on the chemical composition. On the above context, this study was carried out to investigate the leave's phytochemical properties, proximate composition, chemical constituents and antibacterial potency of the methanol leaf extract of *O. gratissimum* plants native to Ado-Ekiti, Nigeria.

MATERIALS AND METHODS

Collection of plant material

The leaves of *O. gratissimum* L. used for this study was purchased in September, 2017 from local farmers at Oba market, Ado-Ekiti, Nigeria and identified at Ekiti State University's Herbarium Ado-Ekiti, Nigeria with a voucher number UHAE 2018/001.

Preparation of plant extracts

The leaves were rinsed and air dried, after which, was milled using an electrical blender to obtain smooth powder substance. Plant extracts were prepared by cold percolation method described by Akinpelu and Onakoya, (2006). 200 g each of the powdered plant sample was soaked in 600 ml of methanol in glass containers with lids. The mixture was kept at room temperature for 2 days to permit full extraction of active components. The mixtures were then filtered using Whatman No 1 filter paper into beakers. The extract was concentrated with a rotary evaporator ((model number RE300DB) to obtain dried mass of crude extract. The crude extract before use was reconstituted with Dimethyl sulphoxide (DMSO).

Phytochemical analysis

The qualitative and quantitative phytochemical screening of the plant leaf extract was by standard phytochemical laboratory procedures as described by Harbone, (1998); Friedman *et al*. (2004); Edeoga *et al*. (2005); Mayuri, (2012).

Proximate Analysis

Proximate composition of the leaf extracts was determined by the method described by AOAC, (2005). All determinations were performed in triplicates.

GC-MS analyses

Gas chromatographic (GC) analyses of the methanol extract essential oil was performed on an Agilent GCMSD apparatus equipped with an Rtx-5SIL MS. The identification of the compounds was by comparing their retention indices and mass spectra with those found in literature (Adams, 2001) and supplemented by Wiley and Quadlib 1607 GC-MS libraries.

Antibacterial activity study

The agar well diffusion method was employed for the antibacterial activities of the plant extracts by the method of Nair *et al*. (2005). Wells of 7 mm in diameter were bored in the agar media previously inoculated with bacterial suspension of McFarland standard. The established inhibition with the leaf extracts was measured and recorded as the degree of sensitivity.

Bacteria species used for the study

The bacteria species used were *Staphylococcus aureus, Pseudomonas aeruginosa, Arthrobacter sanguinis, Vibrio fluvialis, Citrobacter freundii, Staphylococcus epidermidis, Salmonella typhi, Bacillus megaterium, Corynebacterium accolens* and *Bacillus mycoides*. All were obtained from the Department of Biological Sciences, Afe Babalola University Ado-Ekiti, Nigeria. For maintenance and viability, the bacteria species were regularly sub cultured on nutrient agar slants and stored in refrigerator.

Standard antibiotics

Antibiotics disc containing Gentamicin (GEN) 10 µg, Ceftriaxone (CTR) 30 µg, Ceftazidime (CAZ) 30 µg, Cefuroxime (CRX) 30 µg, Cefixime (CFM) 5 µg, Ciprofloxacin (CPX) 5 µg, Nitrofurantion (FT) 30 µg, Erythromycin (ERY) 5 µg, Cloxicillin (CXC) 5 µg, Ofloxacin (OFL) 5 µg and Amoxycillin (AXL) 30 µg were used as references. All tests were performed in duplicate.

Determination of Minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the plant extract was determined by broth dilution method using Mueller Hilton broth. The tubes were incubated at 37ºC for 24 h and observed for turbidity by checking the first tube in the series that showed no visible trace of growth. The first tube in the series with no visible growth after the incubation period was taken as the MIC according to (Okore, 2005).

Determination of minimum bactericidal concentration (MBC)

The MBC was determined by sampling all the macroscopically clear tubes. The tubes were gently mixed and 100 µl of the sample was removed and pour plated with plate count agar (PCA). The plates were allowed to solidify and incubated at 37°C for 24 h. After incubation; the concentration of the extract that did not produce any bacterial growth on the medium was recorded as the MBC values for the extract as the case may be (Irkin and Korukluoglu, 2007). This evidence was matched with the MIC test tube that did not show evidence of growth after 48 hours of incubation.

Statistical analysis

Results were expressed as Mean ± SD. The difference between experimental groups were compared by One-way Analysis of Variance (ANOVA) followed by Dunnet

Multiple comparison test (control vs test) using the SPSS version 16.

RESULTS AND DISCUSSION

The quantitative phytochemical components of *O. gratissimum* extract as presented in Table 1, shows the presence of bioactive compounds of tannins, phenols, saponins, flavonoids, reducing sugars, alkaloids, steroids and cardiac glycosides while terpenoids and cyanogenetic glycosides were absent. Quantitative phytochemical of the leaf extract showed high concentration of 741.5 mg/100g and 346.95 mg/100g for saponins and flavonoids respectively while concentrations of alkaloids, phenols, steroids, tannins, reducing sugars and cardiac glycoside were 137.5 mg/100g, 44.75 mg/100g, 36.67 mg/100g, 16.6 mg/100g, 3.23 mg/100g and 2.37 mg/100g respectively. The results compare well with the phytochemical constituent of *O. gratissimum* leaf extract as reported by Oboh, (2006) but contrary to the reports of Chetia *et al.* (2014), who reported the presence of terpenoids and cyaogenetic glycoside. This may be as a result of habitat differences which play significant role in production of secondary metabolites in plants (Cavaliere, 2009). Herbs that have tannins as a component are astringent in nature and are used for treating intestinal disorder such as diarrhoea and dysentery (Dharmamanda, 2003) while saponins have been reported to reduce haematological parameter (Jimoh *et al*., 2008). Also, the presence of alkaloids which contains quinine, morphine and resperine has amazing effect on humans and has led to the development of powerful pain killer medications (Kam and Liew, 2002).

The proximate analysis of *O. gratissimum* leaf extract is represented in Table 2. The results shows high percentage of carbohydrate (55.76%), protein (18.18%), moisture (11.53%) and crude fibre (8.70%); while ash content (2.71%) and fat content (3.08%) were of low values. The high carbohydrate content constitute a major class of naturally occurring organic compounds which are essential for maintaining plant and animal lives. The plant is a good source of carbohydrate (55.76 mg/100g) when consumed because it meets the Recommended Dietary Allowance (RDA) values (Food and Nutrition Board, 2002). Proteins act as enzymes, hormones, and antibodies. They are important for the formation of bones, teeth, hair and the outer layer of skin and they help maintain the structure of blood vessels and other tissues. *O. gratissimum* is also a good source of protein as it provides more than 12% of the caloritic value from protein as reported by Fagbohun *et al.* (2012). The ash content recorded from the leaf extract was 2.71%, a value lower than what was reported by Fagbohun *et al*. (2012); Chetia *et al.* (2014). The ash content as observed is a reflection of the amount of mineral elements present in the leaf extract. The moisture content of the leaf extracts is relatively high when compared to that reported by Fagbohun *et al*. (2012) as 5.04%; Chetia *et al*. (2014) as 8.96%. The values of the plant's crude fat and crude fibre are 3.08% and 8.70% respectively. These contents were however lower than the values reported by Fagbohun *et al*. (2012) which were 7.57% and 11.38% respectively. Dietary fat increases the tastiness of food by absorbing and retaining flavour. However excess fat consumption is implicated in certain cardiovascular disorders (Antia *et al.*, 2006).

The essential oil extracted from the leaves using methanol contained 20 components made up of both monoterpenes and sesquiterpenes. Data of the essential oil extract is presented in Figure 1 and Table 3. Figure 1 is the chromatogram showing the different peaks while Table 3 shows the constituents of the methanol essential oil. It shows that the main components are Benzene acetic acid, .alpha.-[(diphenymethyl)] (24.52%), 4-Cyclohepta-2,4,6-trienyl-benzoic acid (16.42%), 9-Octadecenoic acid, methyl ester. (E)-(11.92%), Methyl stearate (11.43%), Thymol (4.92%) and 2, 6-Dimethyl-N-(diphenyl-methylene) benzene (4.77%). The other constituents appear in low percentages.

Table 1: Phytochemical compositions of *O. gratissimum* leaf extract

Parameter	Methanol extract	Values (mg/100g)
Alkaloids	+	137.50
Tannins	+	16.60
Phenols	+	44.75
Saponins	+	741.50
Flavonoids	+	346.95
Terpenoids	-	-
Steroids	+	36.67
Cardiac Glycosides	+	2.37
Cyanogenetic Glycosides	-	-
Reducing sugar	++	3.23

Each value is a mean of three replicates. Keys: - = Absent, + = Present, ++ = Moderate

Table 2: Proximate Composition of *O. gratissimum*

Parameters	Mean values (%)
Moisture	11.53±0.15
Ash	2.71±0.07
Fat	3.08±0.05
Crude fibre	8.70±0.09
Protein	18.18±0.02
Carbohydrate	55.76±0.27

Values are expressed as mean ±Standard Error (SE) of the three replicates.

Eugenol, thymol, methyl cinnamate and geraniol have been reportedly used as antimicrobial (Sahoo and Kumar, 2013). *O. gratissimum* secondary metabolites appear to be a source of many biologically active compounds (Unnithan and Undrala, 2017). Gas chromatography mass spectro-photometry has played important role in the identification of the chemical components present in oil extracts. It utilized an apparatus which is used to analyze and define the components of the essential oils using the methods employed by (Sajjadi, 2006; Pripdeevech *et al*. (2010).

The antibacterial activity of the leaf extracts is presented in Tables 4a and 5a alongside the sensitivity of the test organisms to selected standard antibiotics (Tables 4b and 5b). With the three different concentrations of methanol extract, inhibition of all the test bacteria species was established. Among the Gram positive bacteria, *Corynebacterium accolens* was the most inhibited with zones of 31.00, 28.25 and 22.00 mm at concentrations of 100, 50 and 25 mg/ml respectively while *Bacillus mycoides* was the least inhibited with zones of 17.00, 12.75 and 10.00 mm at concentrations of 100, 50 and 25 mg/ml respectively. The essential oil also most inhibited *Corynebacterium*

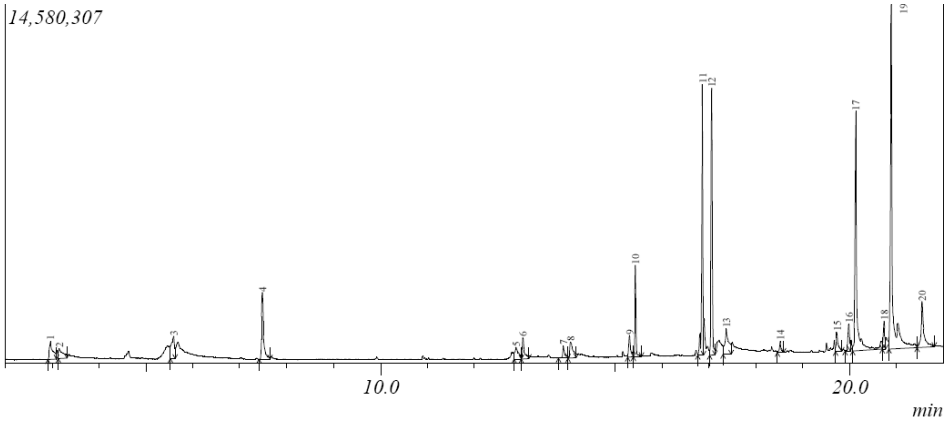

Fig. 1: GC-MS chromatogram of the methanol essential oil extract of *O. gratissimum* leaves.

Table 3: Constituents of the methanol essential oil extract of *O. gratissimum* leaves

Peak	Component	R. T	R. Area %	Height %
1	Benzaldehyde	2.965	2.28	1.16
2	Aniline	3.154	1.61	0.64
3	Benzoic acid	5.589	2.49	1.42
4	Thymol	7.481	4.92	4.24
5	Benzenamine, N-(phenylmethylene)-	12.890	1.42	0.74
6	Benzenamine, N-(phenylmethylene)-	13.036	1.65	1.36
7	Benzenamine, 4-(phenylmethyl)-	13.898	0.89	0.78
8	Benzenamine, 4-(phenylmethyl)-	14.053	1.88	1.02
9	Alpha.,alpha.-Diphenylglycine	15.307	1.48	1.40
10	Hexadecanoic acid, methyl ester, (E)-	15.433	3.62	5.84
11	9-Octadecenoic acid, methy ester. (E)-	16.859	11.92	17.22
12	Methyl stearate	17.059	11.43	16.94
13	Octadecanoic acid	17.370	3.52	1.63
14	Methyl 18-methylnonadecanoate	18.519	0.65	0.72
15	N-Benzoyl-N-benzoyloxy-2-aminofluorene	19.728	1.38	1.26
16	4-Cyclohepta-2,4,6-trienyl-benzoic acid	19.980	1.56	1.74
17	4-Cyclohepta-2,4,6-trienyl-benzoic acid	20.131	16.42	15.23
18	Benzeneacetic acid, .alpha.-[(diphenymethyl)]	20.732	1.58	1.80
19	Benzeneacetic acid, .alpha.-[(diphenymethyl)]	20.888	24.52	21.94
20	2,6-Dimethyl-N-(diphenylmethylene)benzene	21.544	4.77	2.91

Key: R. T= Retention time on the column in minutes. R. Area= relative area (peak area relative to the total peak area).

accolens with zone of 33 mm and least inhibited *Bacillus mycoides* with zone of 24 mm (Table 4a). Among the Gram negative bacteria, *Vibrio fluvialis* was the most inhibited with zones of 19.75, 15.00, 12.50 mm and *Salmonella typhi* the least inhibited with zones of 14.00, 10.75, 10.00 mm at concentrations of 100, 50 and 25 mg/ml respectively. Meanwhile, the essential oil most inhibited *Citrobacter freundii* with zone of 31.18 mm and least inhibited *Vibrio fluvialis* with zone of 23.01 mm (Table 5a)

The result of the antibiotic sensitivity assay on the test bacteria shows that some of the antibiotics were active against the test bacteria species except Cloxicillin (5µg) which had no antimicrobial activity against Gram positive bacteria and Amoxycillin (30 µg) against the Gram negative bacteria (Table 5b). While all employed antibiotics had no antimicrobial effect on *Pseudomonas aeruginosa* (Table 5b), the methanol leaf extract of *O. gratissimum* inhibited this bacterium with zones of 18.25, 15.50 and 10.75 mm at concentrations of 100, 50 and 25 mg/ml respectively (Table 5a).

Some of the Gram positive bacteria species showed higher susceptibility to the plant extract in comparison with the conventional antibiotics. Such bacteria species in this study are *Staphylococcus aureus* and *Corynebacterium accolens* which were inhibited with zones of 29.75 and 31.00 mm at concentrations of 100 mg/ml respectively. Therefore, it can be said that *O. gratissimum* exhibited good antimicrobial activity against the texted bacteria species as compared with the antibiotics. Also, the Gram positive tested bacteria were more susceptible to the plant extracts (Table 4a) than the Gram negatives (Table 5b). This was seen in the zones of inhibition and might be ascribed to the morphological differences between cells making the Gram negative bacteria as highly resistant (Nostro *et al*., 2000). Despite the differences in bacteria cells composition, all the tested bacteria species were however susceptible to the extract at 100, 50 and 25 mg/ml concentrations.

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the plant extract against the ten (10) tested bacteria are shown in Table 6. The minimum inhibitory concentration (MIC) of *O. gratissimum*a on the tested bacteria species was at 25 mg/ml except *Vibrio fluvialis* that only yielded at concentration of 50 mg/ml. The minimum bactericidal concentration (MBC) of *O. gratissimum* was at 50 mg/ml concentration for all except *Vibrio fluvialis and Pseudomonas aeruginosa* that were totally inhibited at concentration of 100 mg/ml. This results correlates with the findings of Osazee *et al*. (2013).

Table 4a: Susceptibility of Gram positive bacteria to plant extract and essential

	O. gratissimum			
Bacteria species	100 g/ml	50 mg/ml Oil	25 mg/ml	Essential
Bacillus mycoides	17.00±1.29	12.75±1.31	10.00±0.40	24.00±0.10
Arthrobacter sanguinis	18.50±1.44	18.00±1.47	14.75±1.18	25.00±0.03
Staphylococcus epidermidis	21.25±0.25	18.25±0.75	17.25±0.47	31.24±0.01
Staphylococcus aureus	29.75±1.10	20.00±0.81	14.75±0.75	33.18±0.21
Bacillus megaterium	20.50±1.50	15.00±0.70	12.75±0.47	31.20±1.04
Corynebacterium accolens	31.00±0.70	28.25±1.10	22.00±1.00	33.20±1.13

Values are recorded in mm = millimetre

Table 4b: Susceptibility of Gram positive bacteria to antibiotics

	Antibiotics							
Bacteria species	Erythromycin 30 µg	Gentamycin 10 µg	Ceftazidime 30 µg	Ceftriaxone 30 µg	Cloxicillin 5 µg	Ofloxacin 5 µg	Amoxycillin 30 µg	Cefuroxime 30 µg
Bacillus mycoides	0.00±0.00	21.00±0.50	0.00±0.00	0.00±0.00	0.00±0.00	21.50±0.00	0.00±0.00	0.00±0.00
Arthrobacter sanguinis	0.00±0.00	14.75±0.25	0.00±0.00	21.50±0.50	0.00±0.00	15.00±0.00	0.00±0.00	0.00±0.00
Staphylococcus epidermidis	0.00±0.00	17.75±0.25	22.75±1.25	27.75±0.25	0.00±0.00	23.50±1.50	14.25±1.25	16.00±0.25
Staphylococcus aureus	0.00±0.00	17.50±0.50	0.00±0.00	21.25±0.25	0.00±0.00	16.25±0.75	0.00±0.00	0.00±0.00
Bacillus megaterium	22.25±0.25	18.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	21.75±0.25	0.00±0.00	0.00±0.00
Corynebacterium accolens	0.00±0.00	14.25±0.75	0.00±0.00	0.00±0.00	0.00±0.00	21.75±0.25	0.00±0.00	0.00±0.00

Values are recorded in mm = millimetre

Table 5a: Susceptibility of Gram negative bacteria to plant extract and essential oil

	O. gratissimum			
Bacteria	100 mg/ml	50 mg/ml	25 mg/ml	Essential Oil
Salmonella typhi	14.00±0.40	10.75±0.25	10.00±0.00	27.15±0.02
Vibrio fluvialis	19.75±0.85	15.00±0.40	12.50±0.00	23.01±0.04
Citrobacter freundii	17.50±0.86	17.25±0.62	15.00±0.00	31.18±1.03
Pseudomonas aeruginosa	18.25±1.31	15.50±0.20	10.75±0.40	27.10±0.04

Values are recorded in mm = millimeter.

Table 5b: Susceptibility of Gram negative bacteria to antibiotics

	Antibiotics							
Bacteria	Gentamycin 30 µg	Ceftazidime 10 µg	Cefuroxime 30 µg	Cefixime 5 µg	Ofloxacin 5 µg	Amoxycillin 5 µg3	Nitrofurantion 30 µg	Ciprofloxacin 5 µg
Salmonella typhi	18.25±0.25	18.00±0.50	13.25±0.25	22.25±0.75	23.75±0.75	0.00±0.00	21.50±0.00	20.75±0.25
Vibrio fluvialis	17.25±0.75	0.00±0.00	14.00±1.00	0.00±0.00	20.75±0.75	0.00±0.00	19.00±1.00	22.75±0.25
Citrobacter freundii	15.75±0.25	18.00±0.00	11.75±0.25	28.00±0.50	22.25±0.25	0.00±0.00	20.25±0.75	23.50±0.00
Pseudomonas aeruginosa	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values are recorded in mm = millimeter

Table 6: Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) (mg/ml) of *O. gratissimum* on test bacteria species

Microorganisms	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	25	50
Salmonella typhi	25	50
Staphylococcus epidermidis	25	50
Bacillus mycoides	25	50
Pseudomonas aeruginosa	25	100
Bacillus megaterium	25	50
Vibrio fluvialis	50	100
Corynebacterium accolens	25	50
Citrobacter freundi	25	50
Artrobacter sanguinis	25	50

Conclusions

From the above results showing the biologically active compounds present in *O. gratissimum*m methanol crude leaf extract, there is scientific basis for the use of this plant as a potent free radical scavenger and as a potential therapeutic agent in treating microbial infections. Also these compounds contained can serve as new potential antibiotic source against antibiotics resistant bacteria species. The quantities of bioactive components detected justify their use as medicinal plants in ethnobotany and may be useful either singly or in combination for therapeutic purposes.

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