Phytochemical Composition and Antibacterial Activities of the Ethyl Acetate Leaf Extract of *Ocimum basilicum*

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Abstract

Introduction

Antibiotic resistance is a serious threat to humankind. There is need for the development of new therapeutic options. *Ocimum basilicum* has been used traditionally as a medicinal herb against infectious diseases and as a food additive.

Objective

This study aimed at evaluating the phytochemical composition and *in vitro* antibacterial activities of the ethyl acetate leaf extract of *Ocimum basilicum*.

Methods

Ocimum basilicum leaves were collected from Mbeere, Embu County, Kenya and ethyl acetate extraction done at Kenyatta University. Phytochemical composition was evaluated by Gas chromatograph mass spectrophotometry while antibacterial activities were evaluated by disc diffusion and broth microdilution methods. The test microorganisms were methicillin resistant *S. aureus, S. aureus, P. aeruginosa* and *E. coli*.

Results

The extract exhibited a broad spectrum antibacterial activity. The gram negative bacteria showed more susceptibility to the organic extract compared to the gram positive bacteria. *Pseudomonas aeruginosa* had the highest zone of inhibition (27.00 ± 2.00 mm), while the *S. aureus* isolate had the lowest zone of inhibition (24.00 ± 1.00 mm). *Escherichia coli, S. aureus* and MRSA species had an MIC of 62.5 mg/ml compared to *P. aeruginosa* with an MIC of 125mg/ml when exposed to the leaf extract. The antibacterial activity could be attributed to the synergistic effects of phytochemicals such as terpenoids, alkaloids, essential oils, fatty acids, flavonoids and aldehydes that have been known to have antimicrobial properties. A total of 30 phytochemical compounds were eluted from the extract of *O. basilicum*. Nootkatone, a sesquiterpenoid had the highest concentration at 20.86mg/g. The compound 1,3-Dimethyl-5-isobutylcyclohexane, a fatty acid had the lowest concentration at 0.10mg/g.

Conclusion

O. basilicum has antibacterial activities on the tested pathogens and can be used to treat infections. Phytochemicals with antibacterial effects in the herb can be used as lead molecules in developing new antibacterial drugs.

Introduction

Infectious diseases account for 41%, measured in Disability-Adjusted Life Years, of the global disease burden. That is far more than injuries (16%) and close to non-infectious diseases (43%) [1]. Among the major causes of this burden is the emergence of antibiotic resistance, a problem that is evolving towards being a global pandemic. It has resulted in the appearance of multidrug resistant bacteria, or superbugs [2, 3]. Antibiotic resistance is a serious public health concern, especially in the developing countries with a high burden of infectious diseases [3]. Pathogens with a significant health threat include Methicilin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*). These species are responsible for a number of diseases including pulmonary infections, urinary tract infections, skin and soft tissue infections, cellulitis, folliculitis and septicaemia [4–8]. The problem posed by these antibiotic resistant pathogens has brought to the fore the need for the development of novel antibacterial agents [3].

Biologically active compounds that could be isolated and harnessed for their antibacterial, antiviral and antifungal activities are found, naturally, in plants. These active compounds include alkaloids, tannins, terpenoids, fatty acids and flavonoids. They are secondary metabolites with complex structures [9, 10]. Their antimicrobial mechanisms include cell wall damage, cytoplasmic membrane damage, formation of reactive oxygen species, Deoxyribonucleic acid fragmentation, phosphatidylserine externalization, metacaspase activation, mitochondrial membrane depolarization, nuclear condensation, modulation of transcription factors, redox signaling and redox-sensitive transcription factors [11]. Exploitation of these factors can help in the development of better antimicrobial approaches. It is, therefore, conceivable that knowledge and data regarding the therapeutic potential of medicinal herbs is of great scientific interest as effective alternatives to the battle of antibiotic resistant microorganisms. Studies into the pharmacological activities of medicinal herbs and plants aim at providing empiric evidence on the use of traditional medicine, their pharmaceutical applications and commercialization of their active components.

Species of the genus *Ocimum* have been studied for their medicinal properties and some have been found to possess anti-inflammatory, analgesic, antidiabeteic, antioxidant and antimicrobial activities [12, 13, 14]. *Ocimum basilicum* is a herb that grows in the sub Saharan Africa, Asia pacific among other regions. Its leaves have been used among the Mbeere people of Kenya to treat infectious diseases [15]. Based on its use in Kenya, it was compelling to determine the antibacterial potential of its leaves on different bacterial species. The phytochemical compositions of this herb was also determined and associated with its antibacterial actions.

Methodology

Plant Material

Ocimum basilicum was obtained from Siakago, Kenya, on longitude 29^{o} and between latitudes 0^{o}35’38”S and 37^{0}38’12”E in January, 2017. Collection was based on its use locally. The plant was taxonomically identified and a voucher specimen deposited at the National Museum herbarium. The leaf samples were

cleaned under running tap water and dried at room temperature to preserve heat sensitive molecules. Dried leaves were then ground to powder using an electric mill.

Extract Preparation

Five hundred grams of the powdered *O. basilicum* leaves were soaked in 1.5 liters of ethyl acetate (LOBA Chemie, Mumbai, India) in a volumetric flask for 72 hrs. The solution was sieved using a muslin cloth and filtered using a Rocker 400 vacuum pump into a conical flask. Concentration to remove the solvent was done by a rotating evaporator. Percentage yield was calculated on a dry weight basis. The extract was stored at 4^{o}C. This procedure adopted that used by Sui *et al.* with minor modifications (16).

$$Percentage\ Yield = \frac{Weight\ of\ Final\ Extract}{Weight\ of\ the\ Soaked\ Sample} \times 100$$

Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was done according to the procedure by Arora and Saini [17]. One gram of the extract was weighed into a 1.5 ml Eppendorf tube and dissolved using 1 ml of ethyl acetate. The mixture was vortexed for 30 seconds and sonicated for five minutes using a Branson 2510E-DTE sonicator. The sample was spun in a centrifuge for 5 minutes at 1300 rpm. The supernatant was transferred into 2ml auto sampler vials and analyzed using a Gas Chromatograph Mass Spectrometry (7683 Agilent Technologies, Inc., Beijing, China).

Antibacterial Assays

Four concentrations of the extract (1 g/ml, 0.75 g/ml, 0.50 g/ml and 0.25 g/ml) were prepared by dissolving in 4% Dimethyl sulphoxide (DMSO). These concentrations were put in sterile bijou bottles and refrigerated at 4^{0}C during use.

Bacterial Strains

Four strains with American Type Culture Collection numbers (ATCC) were used; two Gram negative bacteria (*E. coli* ATCC 29211 and *P. aeruginosa* ATCC 27853) and two Gram positive bacteria (*S. aureus* ATCC 25923 and MRSA ATCC 43300). These were obtained from archived cultures at the Pharmacy, Complementary and Alternative Medicine departmental laboratories, Kenyatta University. Clinical isolates of *E. coli, S. aureus* and *P. aeruginosa* were gifted to me by Mr. Jonathan Mateba of the department of Biochemistry, Microbiology and Biotechnology, Kenyatta University.

Determination of Zones of Inhibition, Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Antibacterial assays were done according to the Clinical and Laboratory Standards Institute guidelines [18]. Assays were done in the Medical Laboratory Sciences departmental laboratories at Kenyatta

University. The test bacteria were sub-cultured on nutrient agar medium, and incubated at 37^{o}C for 18 hours. Using a sterile wire loop, fresh growing colonies were transferred into capped sterile glass tubes containing nutrient broth and incubated at 37^{o}C for 18 hours to form the stock suspensions.

Microbial cultures from the stock suspensions were inoculated onto the surface of Mueller Hinton Agar and incubated for 18 hrs to obtain fresh growing colonies. A fresh growing bacterial colony was picked from a petri dish using a sterilized wire loop, inoculated into 4 ml of sterile peptone water and incubated at 37°C for 4 hrs. The bacterial suspensions were then adjusted in order to obtain turbidity comparable to 0.5 McFarland's standard, which corresponds to about 1-2×10^{8} colony forming units/ml. These suspensions were used within fifteen minutes of preparation.

Determination of antibacterial susceptibility patterns was done by the agar disc diffusion method as per Njeru *et al.* with slight modifications [19]. Gentamycin (40 mcg/ml) and Neomycin (200 mcg/ml) were used as positive controls. Paper discs were prepared from a Whatman filter paper, placed in bijou bottles and sterilized. Aliquots of 0.1 mL of the bacterial suspensions were aseptically pipetted and inoculated by spread plate method on the Mueller hinton agar. The paper discs were impregnated with 20 µl of each concentration of the plant extract and control antibiotics and placed on the surface of the inoculated petri dishes using sterile forceps. Dimethyl sulphoxide at 4% was used as the negative control. Each disk was pressed against the agar medium to ensure level and complete contact. The agar plates were inverted and incubated at 37^{o}C for 24 hrs. After the specified time, diameters of zones of inhibition formed around discs were measured in millimeters (mm) to determine the activity of test samples against different strains. The scale used for determining the strength of antibacterial activities was 8-13 mm: low inhibition; 14-19 mm: moderate inhibition; ≥ 20 mm: high inhibition [20].

Determination of Minimum Inhibitory Concentrations and Minimum Bactericidal Concentration

The broth microdilution method [21] was used to determine the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC). A bacterial colony of each test bacterium was picked using a sterilized wire loop and placed into 4 ml of Mueller Hinton broth medium in test tubes and incubated at 37^{o}C for 4 hours. The bacterial suspensions were then adjusted to 0.5 McFarland's standard. One hundred µl aliquots of the inoculated broth at McFarland's standard were placed into each well of the 96 well microtiter plates. One hundred microliters of the 1 g/ml concentration of the extract was pipetted and added into each of the first well of the inoculated microtiter plate and serial diluted eight times. The extract dilutions obtained ranged from 1 g/ml, to 3.90624 mg/ml. One hundred microliters of gentamycin were pipetted into the control wells and serial diluted to concentrations that ranged from 20 mcg/ml to 0.15625 mcg/ml. Serial dilutions of neomycin were also made. Dimethyl sulphoxide was used as the negative control. The microtiter plates were incubated at 37^{o}C for 24 hours. The concentrations that inhibited growth were taken as the MIC.

For MBC determination, fifty µl aliquots from each inhibited well were pipetted and sero-diluted to the ninth dilution. Fifty µl aliquots of the ninth dilution were pipetted and inoculated onto the surface of the

agar plates. Fifty µl aliquots of the bacterial suspensions in the wells immediately above and below the MIC well were also serial-diluted and inoculated onto the surface of the agar plates. The inoculated plates were incubated at 37^{o}C for 24 hrs. The lowest concentrations that showed complete inhibition of bacterial growth were taken as the minimum bactericidal concentrations.

Data Analysis

The antibacterial experiments were done in triplicates. Statistical analyses were done by One way analysis of variance (ANOVA) and students T-test. Data is presented as Means ± Standard deviation from the mean. Significance was set at $p < 0.05$. Statistical analyses were carried out using Minitab version 17.

Results

Percentage Yield

The percentage yield of *O. basilicum* ethyl acetate leaf extract used in the study was 3.2%

$$Percentage\ Yield = \frac{16.02}{500} * 100 = 3.2\%$$

Phytochemical Concentration in the Ethyl Acetate Leaf Extract of O. basilicum

The GC-MS analysis of the ethyl acetate leaf extract of *O. basilicum* revealed the presence of 30 compounds. Based on the obtained results, Nootkatone, a sesquiterpenoid had the highest concentration at 20.86 mg/g. The compound 1,3-Dimethyl-5-isobutylcyclohexane, a fatty acid had the lowest concentration at 0.10 mg/g. The retention time, molecular formula, the chemical class and the concentrations (mg/g) of the identified compounds in *Ocimum basilicum* leaf extract are presented in Table 1 and Fig. 1 below.

Table 1
Phytochemical Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum*

RT (min)	Phytochemical compound	Molecular Formula	Chemical Class	Concentration (mg/g)
4.9188	Oxirane, 2-methyl-2-(1-methylethyl)-	$C_6H_{12}O$	Fatty acid	0.12
6.6882	9-Thiabicyclo[3.3.1]non-6-en-2-amine, N-methyl-, endo-	$C_9H_{15}NS$	Amine	0.26
8.6367	1H-Imidazole, 2-ethyl-4,5-dihydro-4-methyl-	$C_6H_{12}N_2$	Phenol	0.47
8.9055	1,3-Dimethyl-5-isobutylcyclohexane	$C_{12}H_{24}$	Fatty acid	0.10
9.4654	3,6,6-Trimethyl-cyclohex-2-enol	$C_9H_{16}O$	Phenols	1.96
9.6894	1-Butanol, 3-methyl-, carbonate (2:1) (Diisopentyl carbonate)	$C_{11}H_{22}O_3$	Ester	0.27
11.2572	9,10-Dehydro-6-desoxy-indolinocodeine	$C_{18}H_{19}NO_2$	Alkaloids	0.14
20.1489	Tetradecanal	$C_{14}H_{28}O$	Aldehydes	3.10
22.3886	Tridecenol < 2E->	$C_{13}H_{26}O$	Aldehydes	2.15
22.7021	5-Aminomethyl-5-oxo-1,3,5-diazaphosphorinane	$C_{14}H_{17}N_3O_4$	Amine	0.65
24.4939	Manool oxide	$C_{20}H_{34}O$	Diterpene	0.57
25.5018	2-Methoxyamphetamine	$C_{10}H_{15}NO$	Amphetamine	0.15
26.2185	E-11(13-Methyl)tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	Aldehyde	0.34
26.9352	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	$C_{13}H_{22}OSi_2$		0.18
27.4503	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	Fatty acid	1.73
29.1749	6,3'-Dimethoxyflavone	$C_{17}H_{14}O_4$	Flavanoids	1.82
29.9364	1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl)amino-	$C_8H_{13}N_7$		1.40
30.6531	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	Alkaloid	5.77
31.1235	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	$C_{25}H_{42}$	Triterpene	2.69

RT (min)	Phytochemical compound	Molecular Formula	Chemical Class	Concentration (mg/g)
32.0418	Benzothiophene-3-carboxylic acid, 4,5,6,7-tetrahydro-2-amino-6-ethyl-, ethyl ester	$C_{18}H_{18}ClNO_3S$	Ester	1.44
34.3487	Eicosane, 10-heptyl-10-octyl-	$C_{35}H_{72}$	Essential oils	3.19
35.8269	1H-Pyrazole, 1-(3-methylbutyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-	$C_{14}H_{25}BN_2O_2$	Esters	2.92
39.724	.beta.-Amyrin	$C_{30}H_{50}O$	Triterpene	7.75
40.2391	1,2,4,8-Tetramethylbicyclo[6.3.0]undeca-2,4-diene (Isocaryophyllene)	$C_{15}H_{24}$	Sesquiterpene	6.72
40.7543	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (Nootkatone)	$C_{15}H_{22}O$	Sesquiterpene	20.86
42.1429	Zierone	$C_{15}H_{22}O$	Sesquiterpene	11.45
42.882	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	$C_{12}H_9F_2NO_3$	Alkaloid	0.93
43.0612	9,10-Methanoanthracen-11-ol, 9,10-dihydro-9,10,11-trimethyl-	$C_{18}H_{18}O$	Phenol	0.60
43.3075	A'-Neogammacer-22(29)-en-3-ol, acetate, (3.beta.,21.beta.)- (Lupeol acetate)	$C_{32}H_{52}O_2$	Triterpene	16.76
46.7119	l-Alanine, N-(2-thienylacetyl)-, butyl ester	$C_9H_{14}F_3NO_3$	Phenol	0.25

Antibacterial Activities of the Ethyl Acetate Leaf Extract of O. basilicum

The antibacterial activities of the extract are presented in Tables 2–6. A broad spectrum antibacterial activity was exhibited by the extract of *O. basilicum*. The zones of inhibition ranged from 17.33 ± 0.58 mm to 27.00 ± 2.00 mm in diameter. Gram negative bacteria showed more susceptibility to the organic extract compared to the gram positive bacteria. Among the gram negatives, *P. aeruginosa* had the highest zone of inhibition (27.00 ± 2.00 mm), while among the gram positives, MRSA had the highest zone of inhibition (25.00 ± 1.73 mm) at 1 g/ml concentration of the organic extracts. There was a gradual decrease in the antibacterial activity of the extract that coincided with a decrease in the concentration of the extract.

Table 2
An Antibiogram of Bacterial Strains

Zones of Inhibition (mm)							
Test Agents	MRSA (s)	*S. aureus* (s)	*S. aureus* (i)	*E. coli* (s)	*E. coli* (i)	*P. aeruginosa* (s)	*P. aeruginosa* (i)
Gentamycin	32.33 ± 2.08^{a}	33.00 ± 1.00^{a}	29.00 ± 1.00^{a}	32.00 ± 1.00^{a}	27.00 ± 1.00^{a}	31.33 ± 1.53^{a}	28.67 ± 1.53^{a}
Neomycin	6.00 ± 00^{d}	27.67 ± 0.58^{b}	13.33 ± 0.58^{e}	31.33 ± 0.58^{a}	15.33 ± 0.58^{e}	27.00 ± 1.00^{b}	14.33 ± 2.08^{c}
DMSO	6.00 ± 0.00^{d}	6.00 ± 0.00^{f}	6.00 ± 0.00^{f}	6.00 ± 0.00^{d}	6.00 ± 0.00^{f}	6.00 ± 0.00^{d}	6.00 ± 0.00^{d}
1 g/ml Extract	25.00 ± 1.73^{b}	24.68 ± 0.58^{c}	24.00 ± 1.00^{b}	26.00 ± 1.00^{b}	24.68 ± 0.58^{ab}	27.00 ± 2.00^{b}	26.67 ± 1.53^{a}
0.75 g/ml Extract	23.33 ± 1.53^{b}	23.00 ± 1.00^{c}	21.68 ± 1.53^{bc}	24.68 ± 1.53^{b}	22.68 ± 1.53^{bc}	23.33 ± 0.58^{bc}	21.67 ± 1.56^{b}
0.50 g/ml Extract	22.00 ± 1.00^{b}	21.68 ± 0.58^{d}	20.33 ± 1.53^{c}	22.67 ± 2.52^{bc}	21.33 ± 1.53 ^{cd}	22.33 ± 2.52^{c}	21.33 ± 1.53^{b}
0.25 g/ml Extract	18.00 ± 0.00^{c}	18.00 ± 1.00^{e}	17.33 ± 0.58^{d}	20.00 ± 1.00^{c}	18.67 ± 1.53^{d}	19.67 ± 0.58^{c}	18.67 ± 1.53^{b}

Values are expressed as Mean ± S.D for n = 3. Values with the same superscript letter across columns are not significantly different by One Way ANOVA followed by Tukey's post hoc test for pairwise comparison ($p > 0.05$). Key: s - ATCC strains, i – clinical isolate strains.

Two Sample T tests for the determination of Significance differences between strains

The clinical isolates exhibited a reduced susceptibility to the organic extract compared to the quality control strains. However, this difference was not significant between the ATCC strains and the clinical isolates. A notable reduction in the zones of inhibition was observed in tandem with decreasing concentrations of the extract. These results are presented in Tables 3, 4 and 5.

Table 3
An Antibiogram of *S. aureus*

Zones of inhibition (mm)			
Test Agents	*S. aureus* (s)	*S. aureus* (i)	p. Values
Gentamycin	33.00 ± 1.00	29.00 ± 1.00	0.008
Neomycin	27.67 ± 0.58	13.33 ± 0.58	0.000
1 g/ml Extract	24.68 ± 0.58	24.00 ± 1.00	0.391
0.75 g/ml Extract	23.00 ± 1.00	21.68 ± 1.53	0.295
0.50 g/ml Extract	21.68 ± 0.58	20.33 ± 1.53	0.293
0.25 g/ml Extract	18.00 ± 1.00	17.33 ± 0.58	0.391
Values are expressed as Mean ± S.D for n = 3. Key: s - ATCC strains, i – clinical isolate strains.			

Table 4
An Antibiogram of *E. coli*

Zones of Inhibition (mm)			
Test Agents	*E. coli* (s)	*E. coli* (i)	p. values
Gentamycin	32.00 ± 1.00	27.00 ± 1.00	0.004
Neomycin	31.33 ± 0.58	15.33 ± 0.58	0.000
1 g/ml Extract	26.00 ± 1.00	24.68 ± 0.58	0.139
0.75 g/ml Extract	24.68 ± 1.53	22.68 ± 1.53	0.184
0.50 g/ml Extract	22.67 ± 2.52	21.33 ± 1.53	0.490
0.25 g/ml Extract	20.00 ± 1.00	18.67 ± 1.53	0.295
Values are expressed as Mean ± S.D for n = 3. Key: s - ATCC strains, i – clinical isolate strains.			

Table 5
An Antibiogram of *P. aeruginosa*

Zones of Inhibition (mm)			
Test Agent	*P. aeruginosa* (s)	*P. aeruginosa* (i)	p. values
Gentamycin	31.33 ± 1.53	28.67 ± 1.53	0.099
Neomycin	27.00 ± 1.00	14.33 ± 2.08	0.011
1 g/ml Extract	27.00 ± 2.00	26.67 ± 1.53	0.833
0.75 g/ml Extract	23.33 ± 0.58	21.67 ± 1.56	0.155
0.50 g/ml Extract	22.33 ± 2.52	21.33 ± 1.53	0.598
0.25 g/ml Extract	19.67 ± 0.58	18.67 ± 1.53	0.400
Values are expressed as Mean ± S.D for n = 3. Key: s - ATCC strains, i – clinical isolate strains.			

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

At a concentration of 62.5 mg/ml, the extract exhibited its MIC on *E. coli, S. aureus* and MRSA while on *P. aeruginosa*, MIC was manifested at a concentration of 125 mg/ml. The leaf extract exhibited an MBC of 62.5 mg/ml on the *S. aureus* strain and an MBC of 125 mg/ml on MRSA, *E. coli* and *P. aeruginosa*. These results are presented in Table 6 below.

Table 6
MIC and MBC Values of Different Antimicrobial Agents on Selected Bacterial Strains

	MIC (g/ml)			**MBC (g/ml)**		
Pathogen	Gentamycin	Extract	Neomycin	Gentamycin	Extract	Neomycin
MRSA	0.156	62.5	-	0.156	125.0	-
S. aureus (s)	0.156	62.5	12.5	0.156	62.5	25.0
S. aureus (i)	0.156	62.5	50.0	0.312	62.5	100.0
E. coli (s)	0.312	62.5	12.5	0.312	125.0	25.0
E. coli (i)	0.312	62.5	50.0	0.625	125.0	100.0
P. aeruginosa (s)	0.312	125.0	12.5	0.312	125.0	25.0
P. aeruginosa (i)	0.312	125.0	50.0	1.250	125.0	100.0
Key: s - ATCC strains, i – clinical isolate strains						

Discussion

Medicinal plants contribute to the development of new chemo-preventive agents. It is therefore important to determine their bioactive compounds as well as antibacterial activities [22]. Plants produce a wide collection of phytochemicals that are related to stress, defence mechanisms and antimicrobial activities [23]. The ethyl acetate leaf extract of *O. basilicum* had a variety of phytochemical classes and exhibited antibacterial activities.

Flavonoids are free radical scavengers and water soluble antioxidants with the ability to prevent oxidative cell damage. These metabolites protect the body against cancer, inflammation, allergens, microbes, platelet aggregation, tumors and hepatotoxins [24]. The methylated flavonoid, 6,3'-Dimethoxyflavone exhibits antimicrobial activities. Its derivative has broad spectrum antibacterial activities on *E. coli, P. aeruginosa* and *S. aureus* [25, 26].

The two alkaloids 3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester and Benzo[h]quinoline, 2,4-dimethyl-. Benzo[*h*]quinolones have been used to obtain novel compounds that possess antibacterial activities on *S. aureus*, *Bacillus subtilis*, and *Streptococcus pyogenes*. These compounds are also strong antioxidants, show significant wound healing activities and protect oxidative DNA damage from harmful free radical reactions [27]. Synthetic derivatives of alkaloids are medicinally important for their antispasmodic, analgesic and bactericidal effects. Its physiological activities are apparent when this metabolite is administered in animal models [28].

Terpenoids represent the most diverse and largest class of chemicals among the many metabolites produced by plants. They are a group of compounds possessing an isoprene unit as their basic structure. They are classified based on the number of carbon atoms. Terpenoids provide protection against pathogenic microorganisms [29]. Nootkatone is a sesquiterpene that is synthesized by the oxidation of valencene [30]. Nootkatone has been shown to exhibit antibacterial activities on Gram-positive bacteria including *S. aureus*, *Enterococcus faecalis*, *Corynebacterium diphtheriae, Listeria monocytogenes* and *Bacillus cereus* [30]. This compound exhibits antibacterial effects by targeting metabolites or structures that are specific to Gram-positive bacteria such as the peptidoglycan component of cell walls. Synthetic retinoids contain an isoprene unit that is capable of killing MRSA by penetrating and disrupting the lipid bilayers [31]. There is a possibility that nootkatone inhibits bacterial proliferation by acting on the synthetic pathway of peptidoglycan.

β-caryophyllene is a sesquiterpene, component of dietary regimes that is consumed as a food preservative, flavour or additive and is often referred to as a ‘dietary cannabinoid’ [32]. *β*-caryophyllene has exhibited therapeutic potential due to multiple pharmacological properties such as antioxidant, anti-inflammatory [32] and antimicrobial [33]. Triterpenoids such as *β*-amyrin, a pentacyclic triterpene, are plant constituents with great pharmacological potentials [34]. Lupeol acetate, a pentacyclic triterpene, exhibits a wide spectrum of pharmacological effects including antimicrobial effects [35]. The triterpene 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene was identified in the ethyl acetate leaf extract of

Ocimum basilicum. Manool oxide, a diterpene, has been found to be in abundance in several essential oils, from several plants, which have been shown to have antibacterial activities [36, 37].

Fatty acids identified include Oxirane, 2-methyl-2-(1-methylethyl)-; 11-Eicosenoic acid, methyl ester and 1,3-Dimethyl-5-isobutylcyclohexane. 11-Eicosenoic acid, methyl ester has been associated with antibacterial activity and anti-inflammatory effects [38]. The exact mechanisms by which fatty acids impose their antibacterial effects remain unknown. It has, however, been hypothesized that these molecules induce peroxidative processes that inhibit bacterial fatty acid synthesis. Fatty acids may also interact with cellular membranes thereby causing leakage of molecules from the cells, reduction of nutrient uptake or inhibiting cellular respiration [42]. Amines eluted from the extract include 9-Thiabicyclo [3.3.1] non-6-en-2-amine, N-methyl-, endo-; 1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl) amino- and 2-Methoxyamphetamine. Bicyclic amines have been documented to have antimicrobial and antiparasitic effects [39, 40]. Aldehydes with long chain fatty alcohols such as E-11(13-Methyl) tetradecen-1-ol acetate, Tridecenol < 2E-> and tetradecanal have been shown to have antibacterial activities on *S. aureus* [41]. Tetradecanaal and tridecanol are long chain alcohols that exhibit their antibacterial activity by damaging cell membranes thereby leading to the leakage of K + ions together with subsequent reactions that lead to further leakage [41]. Compounds that possess an alkyl chain promote antibacterial activity and resensitize methicillin susceptible and resistant *S. aureus* to antibiotics [41].

Phenolic compounds with imidazole moieties such as 1H-Imidazole, 2-ethyl-4,5-dihydro-4-methyl-; 3,6,6-Trimethyl-cyclohex-2-enol and l-Alanine, N-(2-thienylacetyl)-, butyl ester possess antibacterial properties. The presence of the imidazole ring to the quinolone moiety increases its antibacterial activity [43]. The antibacterial effects exhibited by the extract were broad spectrum and could be attributed to the effects of these phytochemicals. The inhibitory effects were exhibited in a dose dependent manner with a better efficacy being exhibited on the gram positive bacteria compared to the gram negative. The difference in susceptibility indices could be attributed to the fact that gram negative bacteria have a stable peptidoglycan layer that allows bioactive compounds into the cytoplasm at a lower rate compared to the gram positive bacteria. The dose dependent active nature of the extract on the bacterial strains was due to the decreasing concentrations of active compounds. The ethyl acetate leaf extract of *O. basilicum* was found to have strong antibacterial properties [20].

Conclusions

The results of this study provide an important basis for the use *O. basilicum* in the treatment of bacterial diseases. The extract also contained various pharmacologically active compounds that could be used as lead molecules in the development of new antibacterials. The presence of active chemical groups such as flavonoids, triterpenes, phenols and others in the leaves of *O. basilicum* justify the results obtained. This study also provides an important basis for the use of ethyl acetate extract of *O. basilicum* to control infectious diseases

List Of Abbreviations

O. basilicum Ocimum basilicum

S.aureus Staphylococcus aureus

P. aeruginosa Pseudomonas aeruginosa

E. coli Escherichia coli

MRSA Methicillin resistant *Staphylococcus aureus*

DMSO Dimethyl sulphoxide

ATCC American Type Culture Collection

MIC Minimum inhibitory concentration

MBC Minimum bactericidal concentration

ANOVA Analysis of Variance

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Funding

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests

Authors' contributions

JWR Conceived and designed the study, collected the plant, collected, analysed, interpreted the data and wrote the manuscript. MPN conceived and designed the study, collected the plant, interpreted the data,

substantively revised the manuscript; MM conceived and designed the study, interpreted the data, substantively revised the manuscript; JOO conceived and designed the study, interpreted the data, substantively revised the manuscript .

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Figures

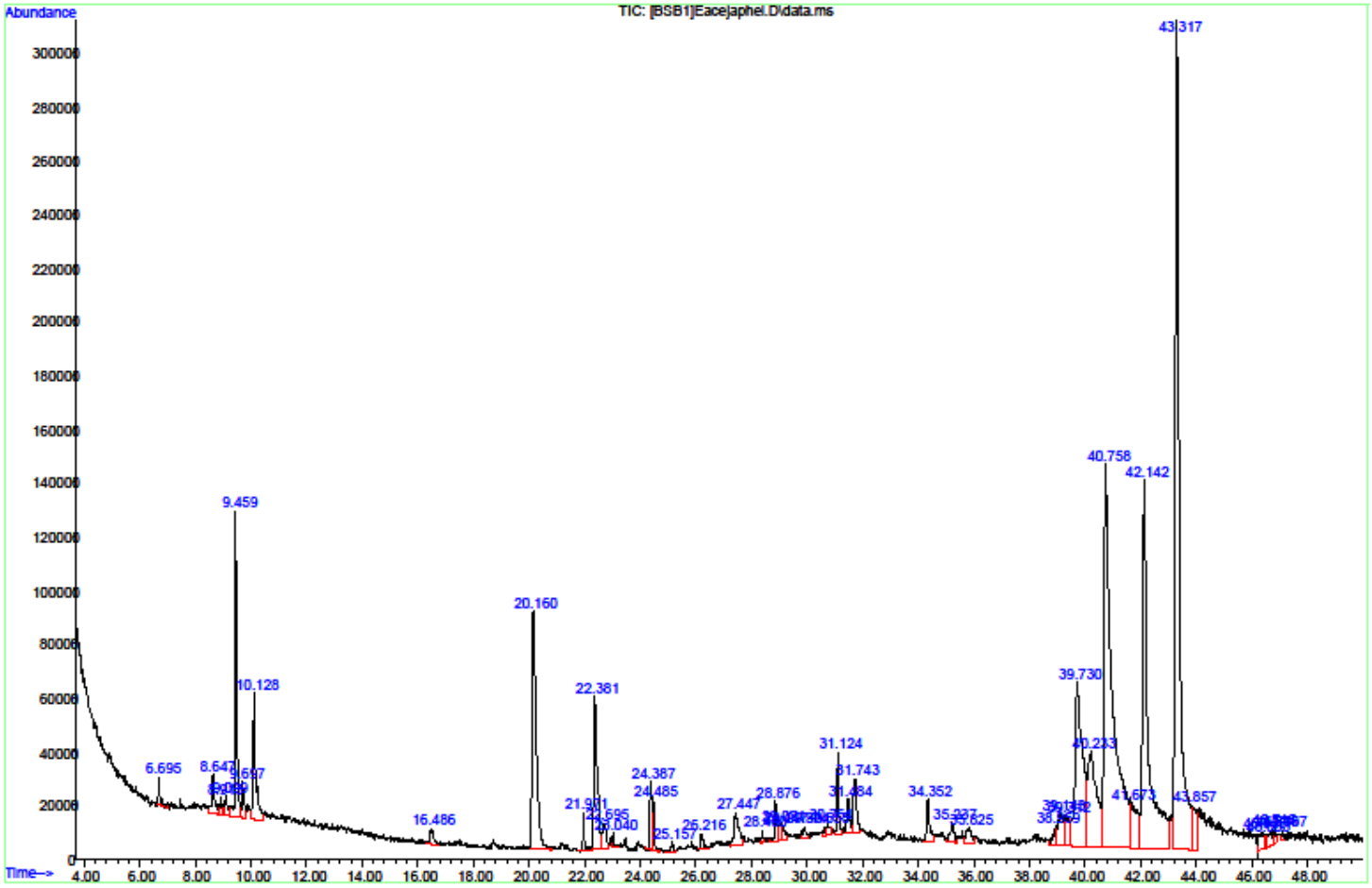

Figure 1

A chromatogram of the ethyl acetate leaf extract of O. basilicum