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Phytochemical Screening and GC-MS Analysis of Methanolic and Aqueous Extracts of *Ocimum kilimandscharicum* Leaves

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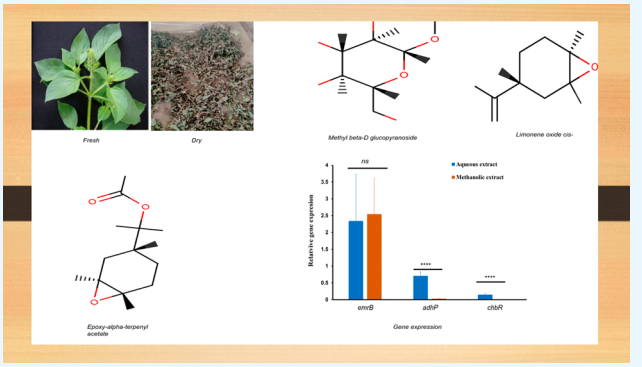
ABSTRACT: Kenyans have long utilized *Ocimum kilimandscharicum*, an East African permanent evergreen plant, to treat measles, stomachaches, diarrhea, mosquito bites (anti-insect), congested chest, cough, and colds. Using conventional qualitative and quantitative techniques, this study was done to identify the secondary metabolites in *O. kilimandscharicum* leaf extracts. The chemical content of the crude extracts from the leaves of *O. kilimandscharicum* has also been investigated and characterized using gas chromatography−mass spectrometry (GC-MS). By using a 1:20 dilution in methanol, in cold maceration, a fine powder of *O. kilimandscharicum* was first extracted then filtered and concentrated after 72 h utilizing a rotary evaporator. By using also a 1:20 dilution in water at 80 °C, a fine powder of *O. kilimandscharicum* was extracted and then filtrated and lyophilized 1 h later. Each extract underwent further gas chromatography−mass spectrometry testing. We found that both extracts contain secondary metabolites such as alkaloids, phenolics, flavonoids, saponins, and tannins. However, the overall amount of phytochemicals in each solvent varied significantly. Total phenolics contents (TPCs) were 5.6 ± 1.20 and 10.8 ± 1.00 mg, total flavonoid contents (TFCs) were 8.2 ± 0.4 and 39.6 ± 2.2 mg, total tannin contents (TTCs) were 0 ± 0.00 and 10.5 ± 0.4 mg, the total alkaloid content (TAC) was 49.2 ± 0.40%, and the total saponin content (TSC) was 38 ± 2.00%. Additionally the gas chromatography−mass spectrometry, revealed a number of high- and low-molecular-weight bioactive molecules at various concentrations for each extract. We also found an inhibitory effect on *adhP* and *chbR* gene expression of *Staphylococcus aureus* and *Salmonella choleraesuius*, respectively. Hence, these chemicals could potentially have a biological and pharmacological significance. Therefore, the discovery of many physiologically active chemicals in the leaf extracts of *O. kilimandscharicum* justifies future biological and pharmaceutical research.



1. INTRODUCTION

Traditional medicines are defined by the World Health Organization (WHO) as knowledge, skills, and practices that vary across cultures and are used to treat or prevent physical or mental illnesses based on theories, first-person experiences, and beliefs.[1] Phytochemicals or active secondary metabolites are abundant in medicinal plants and are widely used in traditional medicine. Only 2% of the known medicinal plants' species (350,659) in the world are scientifically investigated for their phytochemical constituents and beneficial bioactivities.[2,3] Population growth, insufficient drug supply, rising treatment costs, side effects of several synthetic drugs, and the development of resistance to drugs have all contributed to a greater emphasis on the use of plant materials as a source of medicine for a wide range of human ailments. Drugs originating from natural resources play an important role in both traditional and contemporary medical systems[4,5] The World Health Organization (WHO) has taken appropriate efforts to conduct research with the goal of discovering novel and effective therapeutic compounds from plants.[6] Among the many traditionally important plants, the genus *Ocimum sp*. is well-known worldwide and have been used for thousands of years due to its diverse medicinal properties.[7] The genus diversity carries more than 150 individual species. The growth preferences of these plants are mostly located in the tropical and subtropical regions of Asia, Africa, and Central and South America. Many *Ocimum* sp. were investigated for their medicinal properties and use of crude extracts. Specifically, the insecticidal, fungistatic, and antibacterial properties of the plant extracts are well-documented.[8,9]

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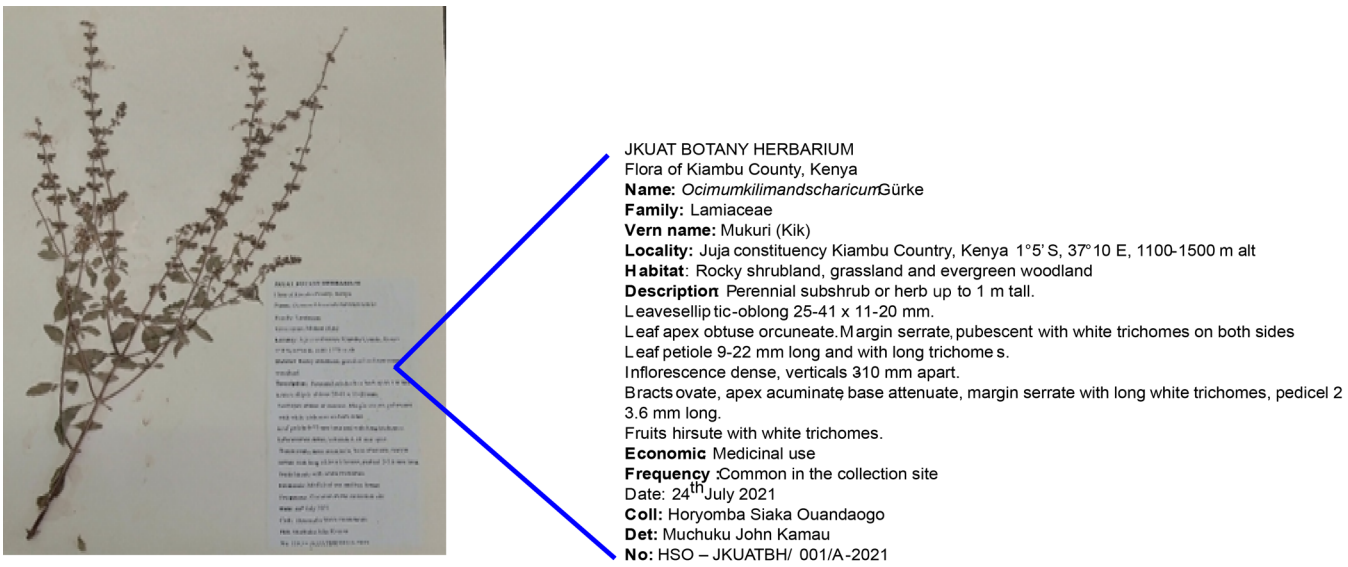

Figure 1. *O. kilimandscharicum* (Herbarium specimen) from H. S. Ouandaogo.

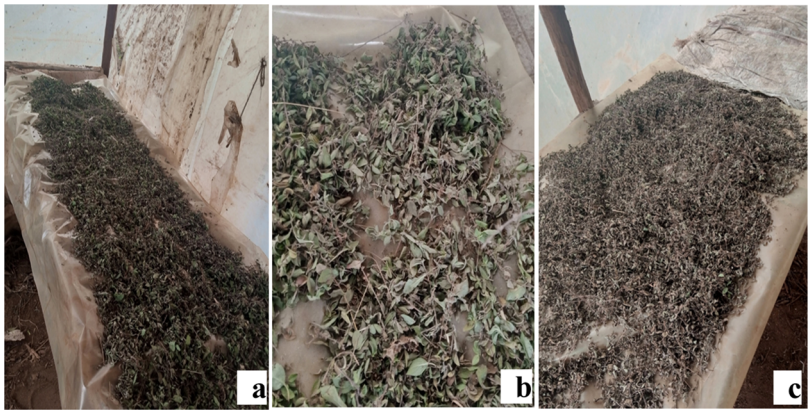

Figure 2. (a–c) Drying *O. kilimandscharicum* leaves from H. S. Ouandaogo.

Table 1. Summary of Target and Reference Primers Used in the Study

bacteria	genes		primers	GC content (%)	*T* (°C)	reference number
Escherichia coli	*emrB*	F	ATTATGACGATTGCGCTGTC	45	56.4	(NP_311575.1)
		R	GTTCCCTGACTGAGTGATGA	50	58.4	
S. choleraesuius	*chbR*	F	AGCTACAGGTTAACGCAACGGAA	48	62.9	(WP_000983393.1)
		R	TCCGGTTAATACCAGGGTAA	45	56.4	
S. aureus	*adhP*	F	GCAGTTGTAACGAAAGATCAC	43	57.4	(WP_001200751.1)
		R	TAACGCCTGTAACATCACC	47	55.2	
	16S_rRNA	27F	AGAGTTTGATCCTGGCTCAG	50	58.4	
		1492R	GGTTACCTTGTTACGACTT	42	53	

From the *Ocimum* genus, *Ocimum kilimandscharicum*, commonly known as camphor basil, is native to Eastern Africa. This species has been extensively used in traditional medicine to cure colds, coughs, malaria, stomach discomfort, and diarrhea.[10] Additionally, *O. kilimandscharicum* is a commercially important fragrant enhancer for cosmetics.[16] Few studies have reported certain biologically active components of *O. kilimandscharicum* from varieties growing in Asia.[11] However, the phytochemical profiling of the East African *O. kilimandscharicum* is still sparse, although its insecticidal and medicinal properties have been documented.[12,13] Currently, comprehensive phytochemical profiling has been done only on few species of the *Ocimum* genus.[14] Additionally, agroecological conditions and cultivation practices could significantly affect the phytochemical profile between plants from different agroclimatic areas.[15] Hence, the characterization of the phytochemicals that are present in *O. kilimandscharicum* cultivated in East Africa (Kenya) needs to be done because of its clinical and medical importance.

To date, numerous extraction, separation, purification, and quantification procedures have been used to identify phytochemicals from medicinal plants. Herein, we used water and alcohol (methanol) extraction coupled with gas chromatographic methods to identify the biologically active compounds present in *O. kilimandscharicum* leaves. We also screened the antibacterial effect of methanolic and aqueous extracts of *O.*

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kilimandscharicum on three strains of bacteria using qPCR. We identified some key secondary metabolites present in aqueous and methanolic extracts and also showed their inhibitory effect on *adhP* and *chbR* gene expression of *Staphylococcus aureus* and *Salmonella choleraesuius*, respectively.

2. MATERIALS AND METHODS

2.1. Chemicals. Legacy Lab Africa Ltd., a Kenyan company, provided all of the chemicals utilized in this investigation. Analytical-grade chemicals were employed throughout.

2.2. Plant Material Collection. In March 2021, *O. kilimandscharicum* leaves were collected from the Juja region in Kenya and transported to the Pan African University of Basic Sciences, Technology and Innovation, Kenya. After collection, the plants specimen were against cross-checked, re-verified and granted a voucher specimen (HSO-JKUATBH/001/A-2021) in the herbarium for reference. Figure 1 shows the herbarium specimen of *O. kilimandscharicum* preserved at JKUAT.

2.3. Preparation and Extraction of Samples. The leaf materials were washed with flowing distilled water, left to air-dry at room temperature (Figure 2), and then crushed into a fine powder using a blender. Briefly, for the methanolic extraction, 100 g of the finely powdered leaves of *O. kilimandscharicum* was put in an orbital shaker incubator at 25 °C with 2000 mL of 70% methanol for 72 h. A rotatory evaporator was used to concentrate the methanolic extract filtrate. Similarly, 100 g of the finely powdered leaves were placed in 2000 mL of distilled water at 80 °C.[17] After 1 h, the mixture was filtrated and lyophilized. A Whatman filter paper number one (1) was used to gravity-filter the resultant combination for both solvents. The percentage yield was computed as below.

$$\text{yield (\%)} = \frac{\text{dry weight of extract}}{\text{dry weight of plant material}} \times 100$$

After that, the extracts were stored at 4 °C for further examination.

2.4. Analysis of Phytochemicals. *2.4.1. Analysis of Qualitative Phytochemical Components.* Using a conventional analytical approach, preliminary qualitative phytochemical analysis was done to determine if secondary metabolites were present or absent in methanol and aqueous leaf extracts.[18,19] For qualitative phytochemical analysis, 0.5 g of plant material extracts was diluted in 100 mL each of methanol and water to provide a stock solution concentration of 5 mg/mL.

2.4.1.1. Test for Alkaloids: Wagner's Test. Each extract was put into a test tube with a volume of around 2 mL. Wagner's reagent was applied, followed by 2 mL of diluted 1% HCL, and incubated for 15 min. Alkaloids are present when a reddish-brown precipitate forms. Iodine (1.3 g) and potassium iodide (2.0 g) were combined to form a stock solution of Wagner's reagent in 100 mL of distilled water. The mixture was placed in an amber container for storage.

2.4.1.2. Test for Tannins. A test tube containing 5 mL of each extract was filled with a few drops of newly made 10% lead acetate. A yellow precipitate implies that tannins are present. 10 mg of lead acetate was added to 100 mL of distilled water to make a 10% solution. This was carried out using a lead acetate test.

2.4.1.3. Test for Saponins: Froth Test. Each extract was diluted in distilled water (1:10 mL) and shaken robustly for 30 s before being incubated at room temperature. After 30 min, a honeycomb foam on top of the surface indicates the presence of saponins.

2.4.1.4. Test for Flavonoids: Alkaline Reagent Test. Two milliliters (2 mL) of weak NaOH was added to 3 mL of each extract in test tubes. Flavonoids are present when a strong yellow coloration develops. 4.25 g of NaOH was dissolved in 50 mL of distilled water to obtain the diluted NaOH.

2.4.1.5. Test for Phenolics. Three drops of this combination were added to 3 mL of each extract, along with a mixture of 10% ferric chloride and 10% ferrocyanide. The precipitate's orange-brown color indicates the presence of phenolics. 10 mg of ferric chloride and 10 mg of ferrocyanide were dissolved in 100 mL of distilled water to create a working solution, and a ferric chloride test was performed.

2.4.2. Quantitative Analysis. Total flavonoid, tannins, and phenolics levels were determined as reported by Madhu et al. and Selvakumar et al.[20,21]

2.4.2.1. Estimation of Total Phenolics Content. With a little modification, the Folin−Ciocalteu technique was used to measure the content of phenolic chemicals in the extracts. In a volumetric flask (10 mL), 50 μL of the sample extracts were combined with 0.5 mL of (1 N) Folin−Ciocalteu reagent. 2.5 mL of 5% sodium carbonate was added after 5 min, and distilled water was used to get it up to the volume of 4 mL. The tubes were incubated for 40 min at room temperature in a dark area with a series of gallic acid standard solutions (2.5, 5, 7.5, 10, and 12.5 mg/mL) made in the same way. The absorbance was measured at 725 nm. The total phenol content was expressed as milligrams of gallic acid equivalent per gram of dried extract. The same method was used to make a blank reagent that was devoid of extracts.

2.4.2.2. Estimation of Total Flavonoid Content. Utilizing aluminum chloride, a colorimetric assay was used to calculate the total flavonoid content. For the reaction, a volumetric flask (10 mL) was filled with 0.5 mL of crude extract, distilled water (0.5 mL), and 5% sodium nitrite (0.15 mL); 10% aluminum chloride (0.15 mL) was added after 5 min. In addition, 6 min after incubation, 2 mL of sodium hydroxide (1 M) was added to the mixture, which was then promptly topped up with 5 mL of distilled water. In the same way, as previously described, a set of standard solutions of rutin (8, 16, 24, 32, and 40 mg/mL) were created. The test tubes were vortexed and kept at room temperature for 30 min in the dark. The absorbance of the test and standard solutions was evaluated using a UV−visible spectrophotometer and reagent blank at a 510 nm wavelength. The findings of the total flavonoid content were represented as rutin equivalents (mg RE/g of dried extract). The same procedure was used to create a reagent blank without extracts.

2.4.2.3. Estimation of Total Tannin Content. Two (2 mL) Eppendorf tubes were filled with 100 mg of poly(vinyl polypyrrolidone) (PVPP). 500 μL of distilled water and the plant sample were added and incubated for 4 h at 4 °C. The Eppendorf tubes were centrifuged at 3000 rpm for 10 min at 4 °C following incubation. Only the nontannin phenolics are present in the supernatant.

The Folin−Ciocalteu technique, with a few adjustments, was used to calculate the tannin content. A 10 mL volumetric flask was filled with distilled water, and 0.1 mL of nontannin phenolics extract was added. Dilution with distilled water

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Table 2. Crude Extract from Leaves of *O. kilimandscharicum*

solvents	initial mass (g)	mass (g)	mass (%)	color	texture
methanol	100	3	3	greenish	sticky mash
water	100	3.2	3.2	brownish	dry powder

Table 3. *O. kilimandscharicum* Leaves' Qualitative Profile of Phytochemicals[a]

solvent	phenolics	flavonoids	tannins	alkaloids	saponins
methanol	+	+	+	+	+
water	+	+	−	+	+
type of test	ferric chloride	alkaline reagent	lead acetate	Wagner's reagent	Froth test

[a]+ = present and − = absent.

Table 4. *O. kilimandscharicum* Leaf Extracts' Total Flavonoids, Phenolics, and Tannins contents

solvent extract	total flavonoids content (mg RE/g extract) ± SE	total phenolics content (mg GAE/g extract) ± SE	total tannins content (mg TAE/g extract) ± SE
methanol	8.2 ± 0.4^{c}	10.8 ± 1.00^{b}	10.5 ± 0.4^{b}
water	39.6 ± 2.2^{a}	5.6 ± 1.20^{d}	0.00 ± 0.00^{e}
mean	23.9 ± 22.20	8.2 ± 3.67	5.25 ± 7.47
F-value	591.58	33.25	75.00
P-value	<0.0001	<0.005	<0.005
LSD (0.05)	3.58	2.50	3.37
CV, %	2.78	2.77	2.78

Values are the mean ± SE. Values with the different superscript letters designate significant differences.

brought the final volume to 4 mL after adding 0.5 mL of (1 N) Folin−Ciocalteu reagent and 2.5 mL of 5% sodium carbonate solution.

Standard tannic acid dilutions (2.5, 5.0, 7.5, 10.0, and 12.0 mg/mL) were made in the same way described above. After vigorous shaking, the mixture was incubated at 30 °C for 40 min.

The absorbances of reference and test solutions were measured with a blank UV−visible spectrophotometer at 725 nm. The nontannin concentration was represented as tannic acid equivalents per gram of dry extract. The blank solution was prepared in a similar way as explained above. The sample's tannin content was then determined using the following equation: tannins (g) = total phenolics (g) − nontannin phenolics (g).

2.4.2.4. Estimation of Total Alkaloid Content. The approach given by Ibibia et al.[22] was modified to estimate the total alkaloid content. 2 g of dried leaf powder was put into a beaker filled with 200 mL of 10% acetic acid and 10 mL of 99.8% ethanol. A Whatman filter paper number one was used to filter the extract after it had been at room temperature for 5 h, and the filtrate concentrated to a quarter of the original volume. Precipitates produced after 10 drops of ammonium were applied to the filtrate one drop at a time. After discarding the supernatant, the precipitates were washed with 15 mL of ammonia solution and oven-dried. A percentage was used to represent the overall alkaloid content.

2.4.2.5. Estimation of Total Saponin Content. The approach given by Ezeonu et al.[23] was modified to estimate the total saponin content. 2 g of dried leaf powder was cooked in a water bath for 2 h using 150 mL of 20% ethanol. 20 mL of diethyl ether was put into a separating funnel after filtering, evaporating, shaken, and recovered. For purification, 60 mL of *n*-butanol was used and dried in the oven. A percentage was used to represent the overall saponin content.

2.4.2.6. Real-Time Quantitative Polymerase Chain Reaction (qPCR). Briefly, the PURE LINK RNA mini kit was used for RNA extraction from bacteria, followed by cDNA synthesis using the FIRE Script RT cDNA synthesis kit. The bacterial wall was lysed and homogenized, followed by the addition of absolute cold ethanol to precipitate the nucleic acids. The RNA was then quantified, and the quality was checked by using a Nanodrop ND-2000 spectrophotometer. The cDNA concentration was adjusted to a baseline of 100 ng/μL for comparison. The Quantitative real-time polymerase chain reaction was used to evaluate the effect of silver nanoparticles of *O. kilimandscharicum*'s methanolic and aqueous leaf extract on gene expression profiles of *emrB*, *adhP*, and *chbR* genes. The quantification cycle, also known as the threshold cycle (Ct), was calculated, and relative mRNA expression levels of target genes were normalized to the housekeeping gene (16S_rRNA) using the $2^{-\Delta\Delta Ct}$. Table 1 shows the primers we used, their GC content, and their reference number.

2.5. GC-MS Analysis. Shimadzu's GC-MS QP2010 series with electron impact ionization mode and a Bpx5 GC column (length, 30 m; thickness, 0.25 m; diameter, 0.25 mm) were used to analyze the samples. Helium gas was used as carrier gas (99.999%) at a constant flow rate of 1 mL/min and an injection volume of 1 μL in size ratio of 10:1. The ion source temperature was 200 °C, while the injector temperature was 250 °C. The oven temperature progressed from 60 °C, maintained for 1 min, to 300 °C and stayed elevated for 30 min at a rate of 15 °C/min. The solvent delay was adjusted from 0 to 45 min, and the mass spectrophotometer was configured in positive electron ionization mode with an ionization energy of 70 eV. A scan interval of fragments from m/z 35 to 500 Dm was fragmented. The peak area/total peak area ratio was used to compute the relative percentage of each component. Lab Solution was the program utilized, while NIST Coin 4.0 (National Institute of Standards Technology) served as the library.

2.6. Statistical Analysis. The mean ± standard error (SE) was used to express experimental results. R studio, Excel, and

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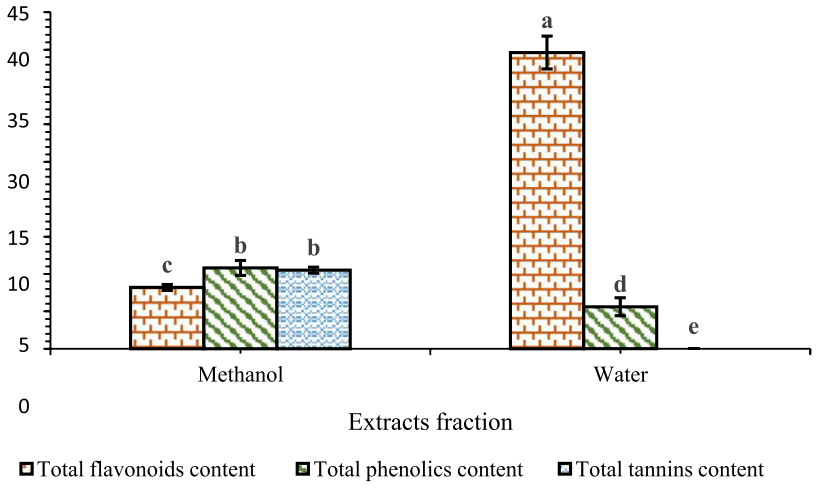

Figure 3. Evaluated total flavonoids, phenolics, and tannins content of the two fractions of *O. kilimandscharicum* leaves. Data standard error (SE) is shown by error bars. Letters indicate significant differences between means.

SAS9.2 were used to perform an analysis of variance (ANOVA) to examine the significance of variation between treatment means. The Tukey multiple comparison test (TMCT) was used to compare the treatment means at a significant level of $P < 0.05$.

3. RESULTS

3.1. Qualitative Phytochemical Analysis. Table 2 summarizes the mass of crude extracts and the percentage mass from *O. kilimandscharicum* leaf material extraction using methanol and water. The yield of the extracts postextraction ranges from 3 to 3.2 g, and the percentage mass of crude extracts ranged from 3 to 3.2%. We can notice that they are almost similar. So, we obtained almost the same mass postextraction and percentage of mass for methanol and water extract. Green and brown pigmentations were observed across the methanol and water extracts. Additionally, the crude extracts were either viscous or dry mash (Table 2).

All of the extracts included phenolics, flavonoids, alkaloids, and saponins, as shown in Table 3. Only the methanolic extract revealed that tannins were present.

3.2. Quantitative Phytochemical Analysis. *3.2.1. Total Phenolics Content (TPC).* The gallic acid standard calibration curve was obtained ($y = 0.1037x + 0.1309$, $R^2 = 0.968$). The findings of the total phenol content were represented as gallic acid equivalent (mg of GAE/g of dried extract). The data were presented as mean ± SE of triplicates, as shown in Table 4. The water extract had the lowest total phenolic content (5.6 ± 1.2 mg GAE/g), whereas methanol extract had the highest (10.8 ± 1.00 mg GAE/g). ANOVA ($F(1,4) = 33.25$, $P < 0.005$) in Table 4 and Figure 3 revealed that there was a statistically significant difference between the groups. The superscripted letters in Table 4 indicate that the TPC values of the different extracts were significant statistically, as determined by the Tukey post hoc test. At the 0.05 level, the mean difference is statistically significant.

3.2.2. Total Flavonoid Content (TFC). The rutin standard calibration curve was generated ($y = 0.0091x + 0.0324$, $R^2 = 0.9645$). The findings of the total flavonoid content were represented as rutin equivalents (mg RE/g of dried extract). The data were presented as mean ± SE of triplicate, as shown in Table 4. The methanol extract had the lowest total flavonoid content (8.2 ± 0.4 mg RE/g), whereas the water extract had the highest (39.6 ± 2.2 mg RE/g). ANOVA ($F(1,4) = 591.58$, $P < 0.0001$) in Table 4 and Figure 3 revealed that there was a statistically significant difference between the groups. The superscripted letters in Table 4 indicate that the TFC values of the different extracts were significant statistically, as determined by the Tukey post hoc test. At the 0.05 level, the mean difference is statistically significant.

3.2.3. Total Tannin Content (TFC). The tannic acid standard calibration curve was generated ($y = 0.1024x + 0.1023$, $R^2 = 0.9962$). The findings of the total tannin content were represented as tannic acid equivalent (mg of TAE/g of dried extract). The data were presented as mean ± SE of triplicate, as shown in Table 4. The analysis has shown that only the methanol extract has tannin content (10.5 ± 0.4 mg TAE/g). ANOVA ($F(1,4) = 33.25$, $P < 0.005$) in Table 4 and Figure 3 revealed that there was a statistically significant difference between the groups. The superscripted letters in Table 4 indicate that the TTC values of the different extracts were significant statistically, as determined by the Tukey post hoc test. At the 0.05 level, the mean difference is statistically significant.

3.2.4. Total Alkaloid and Saponin Content. The findings of the total alkaloids and saponin content were represented as content (%) in the leaf. The data were presented as mean ± SE of triplicate, as shown in Table 5. The analysis has shown that the leaf powder has more alkaloids (49.2 ± 0.40%) than saponins (38 ± 2.00%). ANOVA ($F(1,4) = 90.46$, $P < 0.005$) in Table 5 and Figure 4 revealed that there was a statistically

Table 5. Total Alkaloid and Saponin Contents of *O. kilimandscharicum* Leaf Powder

phytochemicals	content (%) in leaf powder ± SE
alkaloids	49.2 ± 0.40^{g}
saponins	38 ± 2.00^{h}
mean	43.60 ± 7.91
F-value	90.46
P-value	<0.005
LSD (0.05)	3.27
CV, %	2.78

Values are the mean ± SE. The superscript letters indicate significant differences between means.

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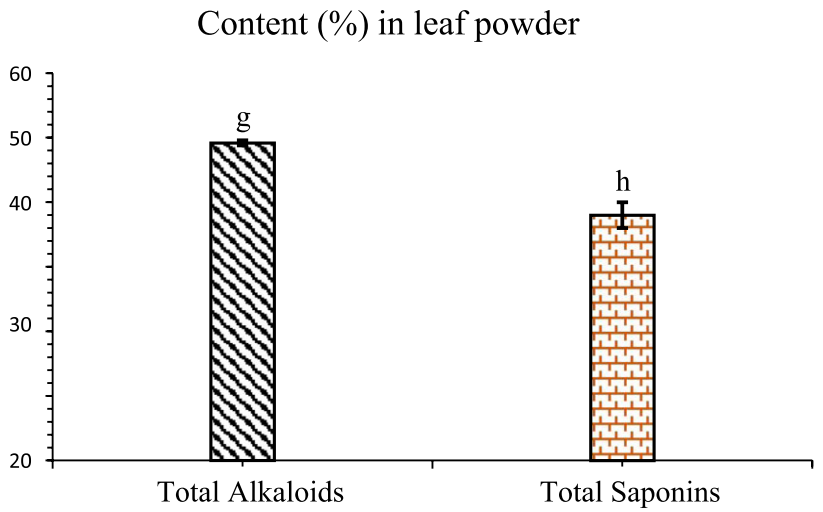

Figure 4. Evaluated total alkaloid and saponin content in *O. kilimandscharicum* leaf powder. Data standard error (SE) is shown by error bars. Letters indicate significant differences between means.

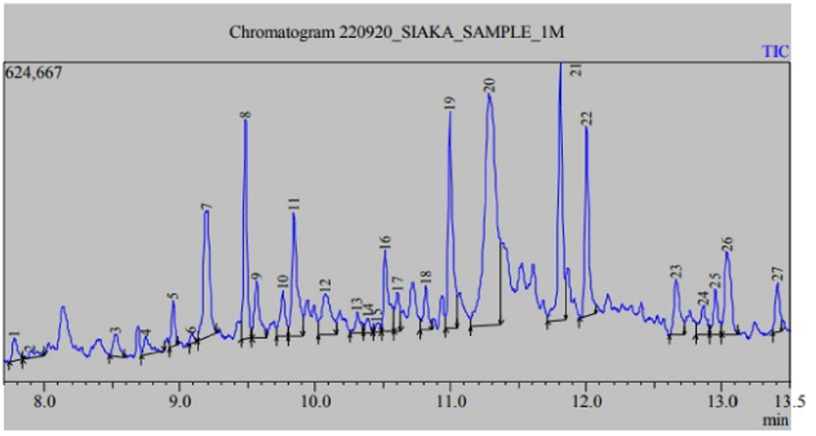

Figure 5. GC-MS chromatogram of the *O. kilimandscharicum* methanol extract.

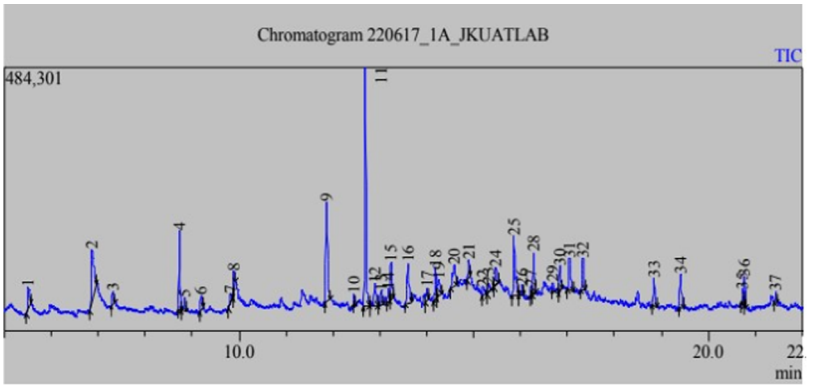

Figure 6. GC-MS chromatogram of the *O. kilimandscharicum* aqueous extract.

significant difference between the groups. The superscripted letters in Table 5 indicate that the TAC and TSC of the leaf powder were statistically significant, as determined by the Tukey post hoc test. At the 0.05 level, the mean difference is significant.

The GC-MS chromatograms of the methanolic and aqueous extracts (Figures 5 and 6), which display the GC-MS profiles of the detected chemicals and Tables 8 and 9 show the phytochemical activity and the structures of some of the identified compounds, respectively. The chromatogram's peaks were combined and then compared to a database of known component spectra kept in the GC-MS NISP library. The methanolic and aqueous extracts of *O. kilimandscharicum* leaves underwent phytochemical analysis using GC-MS. The results showed the presence of various fatty acids, heterocyclic compounds, and esters, among other things. The methanolic extract produced 27 peaks, while the aqueous extract produced 37 peaks.

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Table 6. Some Secondary Metabolites in *O. kilimandscharicum* Methanol Extract Were Identified Using GC-MS and the NIST 17 Spectral Database

sl. no.	RT	name of the compound	molecular formula	MW	% peak area	class
1	7.890	epoxy-α-terpenyl acetate	$C_{12}H_{20}O_2$	196	0.44	monoterpenoids
2	8.745	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.81	alcohol/phenol
3	9.080	epoxy-linalool oxide	$C_{10}H_{18}O_2$	186	0.30	tetrahydrofuran
4	9.840	limonene oxide	$C_{10}H_{16}O$	152	5.50	alkene
5	10.450	DL-isopulegol	$C_{10}H_{18}O$	154	0.36	alcohol
6	10.606	3-ethoxy-*p*-menth-1-en-8-ol	$C_{12}H_{22}O_2$	154	1.43	monoterpenoids
7	10.813	α-methyl-α-4-methyl-3-pentenyl oxiranemethanol	$C_{10}H_{18}O_2$	170	1.69	tertiary alcohols
8	10.992	*trans*-linalool oxide (furanoid)	$C_{10}H_{18}O_2$	170	8.30	tetrahydrofuran
9	11.281	β-D-glucopyranoside	$C_6H_{14}0_7$	180	23.40	O-glycosyl
10	11.807	α-campholenal	$C_{10}H_{16}O$	152	9.23	aldehyde
11	12.950	methyl dihydroisosteviol	$C_{12}H_{34}O_3$	334	1.86	diterpene

Table 7. Some Secondary Metabolites in *O. kilimandscharicum* Aqueous Extract Were Identified Using GC-MS and the NIST 17 Spectral Database

sl. no.	RT	name of the compound	molecular Formula	MW	% peak area	class
1	5.502	DL-glyceraldehyde dimer	$C_6H_{12}O_6$	180.16	2.52	aldotriose/aldehydes
2	6.861	glycerin	$C_3H_8O_3$	92.09	4.00	aldoses/aldehyde
3	7.312	2-hydroxy-γ-butyrolactone	$C_4H_6O_3$	102.09	2.79	ketone
4	8.838	benzenemethanol	C_7H_8O	108.13	2.25	alcohol
5	9.188	2-butanol	$C_4H_{10}O$	74.12	2.16	alcohol
6	9.817	butanoic acid	C_4H_8O	88.11	2.15	carboxylic acid
7	9.817	2,3-dimethyl-, ethylester	$C_8H_{16}O_2$	144.21	2.15	ester
8	11.864	caprolactam	$C_6H_{11}NO$	113.16	2.39	
9	12.692	triacetin	$C_9H_{14}O_6$	218.21	1.81	triacylglycerols
10	13.029	epoxy-linalool oxide	$C_{10}H_{16}O_3$	184.23	2.07	organoheterocyclic compounds
11	13.237	epoxy-α-terpenyl acetate	$C_{12}H_{20}O_2$	196.29	2.25	monoterpenoids
12	14.184	2,6-octadiene-1,8-diol	$C_{10}H_{18}O_2$	170.13	2.37	alcohol
13	14.256	acetin, bis-1,3-trimethylsilyl ether	$C_{11}H_{26}O_4$	278.50	3.34	ethers
14	14.585	imidazole	$C_3H_4N_2$	68.077	4.58	heterocyclic
15	15.313	hexanoic acid, hexyl ester	$C_{12}H_{24}O_2$	200.32	3.27	esters
16	15.465	oxiranemethanol	$C_{10}H_{18}O_2$	150.17	2.40	alcohol
17	15.860	2-furanmethanol	$C_5H_6O_2$	98.1	2.90	alcohol
18	16.276	diethyl phthalate	$C_{12}H_{14}O$	222.24	1.94	ester
19	16.683	megastigmatrienone	$C_{13}H_{18}O$	190.28	2.55	cyclohexanone/ketone
20	19.404	1,2-benzenedicarboxylic acid	C_8H_6O	166.13	2.33	carboxylic acid
21	21.436	fumaric acid	$C_4H_4O_4$	116.07	1.67	carboxylic acid

The detailed tabulations of GC-MS analysis of the methanolic and aqueous extracts are given, respectively, in Tables 6 and 7. Twenty-seven (27) compounds have been elucidated from the analysis of the methanolic extract, of which 11 compounds were effectively matched and identified. 37 compounds have been elucidated from the analysis of the aqueous extract, of which 21 compounds were effectively matched and identified. So, we have for the methanolic extract β-D-glucopyranoside (peak area 23.40%), α-campholenal (peak area 9.23%), *trans*-linalool oxide (furanoid) (peak area 8.30%), limonene oxide cis- (peak area 5.50%), whereas the remainder had a peak area composition of less than 2%. For the aqueous extract, we have imidazole (peak area 4.58%), glycerin (peak area 4%), acetin, bis-1,3-trimethylsilyl ether (peak area 3.34%), hexanoic acid, hexyl ester (peak area 3.27%), 2-furanmethanol (peak area 2.90%), 2-hydroxy-γ-butyrolactone (peak area 2.79%), megastigmatrienone (peak area 2.55%), DL-glyceraldehyde dimer (peak area 2.52%), and oxiranemethanol (peak area 2.40%), whereas the remainder had a peak area composition of less than 2.4%.

4. DISCUSSION

Plants are a plentiful source of bioactive substances that have the potential to be helpful and may be utilized to create novel chemotherapeutic medicines. In traditional and modern medicine, they are used to cure several illnesses and manage various pathogenic agents. Researchers worldwide are looking into the use of pharmacologically active substances obtained from medicinal plants. Herbal medicines are used by 80% of the world's population due to their high efficacy, low cost, non-narcotic nature, and lack of side effects.[24] Phytochemical compounds are classified as secondary metabolites that occur naturally in plants. To date, enormous progress has been made in the identification and functional characterization of bioactive compounds of medical importance.[14] The phytochemical screening approach includes qualitative and quantitative chemical class profiling of ethnomedicinal plant species. Methanolic and aqueous extraction coupled with gas chromatography−mass spectrometry (GC-MS) has been widely used to identify phytochemicals of clinical importance.[18,25]

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Table 8. Summary of the Phytochemical Activity of Some of the Identified Compounds

sl. no.	name of the compound	class	phytochemical activity
1	epoxy-α-terpinyl acetate	monoterpenoids	antibacterial, antioxidant, antitumor, analgesic, anti-inflammatory fungicide, antipyretic, antifeedent
2	2-methoxy-4-vinylphenol	alcohol/phenol	antimicrobial, antioxidant and cytotoxicity activities
3	epoxy-linalool oxide	tetrahydrofuran	antimicrobial and antioxidant
4	limonene oxide cis-	alkene	antioxidant and antimicrobial activity
5	DL-isopulegol	alcohol	antimicrobial, antioxidant and cytotoxicity activities
6	3-ethoxy-*p*-menth-1-en-8-ol	monoterpenoids	antibacterial, antioxidant, antitumor, analgesic, anti-inflammatory fungicide, antipyretic, antifeedent
7	oxiranemethanol, 3-methyl-3-(4-methyl-3-pentenyl)-	tertiary alcohols	antibacterial and antioxidant activities
8	*trans*-linalool oxide (furanoid)	tetrahydrofuran	antimicrobial activity
9	methyl β-D-glucopyranoside	O-glycosyl	antibacterial and antifungal
10	α-campholenal	aldehyde	antimicrobial, antioxidant and cytotoxicity activities
11	methyl dihydroisosteviol	diterpene	antibacterial and antifungal activities
12	thunbergol	diterpene	antimicrobial
13	platydesmine	alkaloid	antibacterial
14	2,2,6 trimethyl 2H,5H,6H pyrano[3.2]quinolin-5-one	sesquiterpenes	antimicrobial
15	ethyl 2-methylallyl ester	fatty acid esters	antibacterial
16	corymbolone	sesquiterpenoid	antiplasmodial
17	*cis*-Z-α-bisabolene epoxide	sesquiterpene	anti-inflammatory, antimicrobial and antioxidant activities
18	bicyclo[4.4.0]dec-2-ene-4-ol	terpene	antibacterial
19	isoaromadendrene epoxide	terpene	antibacterial
20	benzoic acid	aromatic carboxylic acid	antibacterial

Using this approach, we identify the most important bioactive compounds present in *O. kilimandscharicum* leaves. We found the presence of alkaloids, phenolics, flavonoids, and saponins in both aqueous and methanolic leaf extracts of *O. kilimandscharicum*. These results are consistent with prior findings reported in others ocimum species by Singh et al.[26] except for the presence of saponins and alkaloids. However, the presence of these bioactive compounds has been reported in other *Ocimum* species.[27,28] On the other hand, our findings suggest the absence of tannins in aqueous leaf extract, which is not surprising given that the content of tannins are optimal in hydro-alcoholic extracts.[29] Overall, we found a high content of flavonoids in the aqueous extract compared with the alcoholic extract.

The efficiency of a solvent in phytochemical extraction offers an early indication of medicinal quality, whereas quantitative determination is a significant technique to define the standard of a crude drug.

The presence of alkaloids, saponins, tannins, phenolics, and flavonoids in the *O. kilimandscharicum* leaf extract confirmed the pharmacological importance of this plant. Alkaloids have antibacterial, antiviral, anticancer, antifungal, and antimalarial properties, while saponins have insecticidal, anthelmintic, anticancer, antiviral, antibacterial, and antifungal properties. Indeed, essential oils from the East African cultivars of *O. kilimandscharicum* have shown insecticidal properties.[12] Similarly, flavonoids also have antibacterial, antiaging, antiallergenic, anti-inflammatory, anticancer, and antiviral activities, while phenolic compounds have antioxidant and antibacterial properties. Since we observed a large variation in quantities of the phytochemical content between aqueous and alcoholic extracts, we also hypothesized an eventual difference in their pharmacological effect. To test this hypothesis, we evaluated the antibacterial activity of the two extracts on three bacterial strains: *E. coli* (*emrB*), *S. choleraesuius* (*chbR*), and *S. aureus* (*adhP*). We found that the expression of the *E. coli* gene *emrB* was not affected by any of the extracts (Figure 7). However, the expression of the *chbR* gene from *S. choleraesuius* and the *adhP* gene from *S. aureus* was significantly inhibited. Similarly, the ethanol extract of *Ocimum gratissimum* leaves showed higher antibacterial activity than the aqueous extract.[27] Also, the *Ocimum basilicum* ethanol extract revealed a high inhibitory effect against *E. coli* and *S. aureus*[30] Although, we did not find significant inhibitory effect against *E. coli* in the present study. This dissimilarity could be eventually explained by the solvent used for the extraction and concentration use in the antibacterial bioassay. Furthermore, several studies have demonstrated that the extract of *O. basilicum* presents an antimicrobial activity on several Gram-negative and Gram-positive including *E. coli* and *S. aureus*.[31−33] The methanolic extract was more efficient than the aqueous extract (Figure 7). We argue that the difference in antibacterial activities between the methanol and aqueous extracts could be partially linked to the abundance of tannic compounds in the methanolic extract. There is scientific evidence that tannins have antibacterial effects on many human gastrointestinal pathogens including bacteria.[34,35] Traditionally, *O. kilimandscharicum* extracts are used to cure several illnesses such as colds, coughs, abdominal pains, and diarrhea, which are most often caused by pathogenic bacteria. Hence, our results provide scientific evidence of the medicinal importance of this plant.

The GC-MS analysis highlighted 27 compounds in the methanolic extract, and 11 compounds were effectively matched and identified. Thirty-seven compounds were found in the aqueous extract with 21 compounds effectively matched and characterized. Overall, these compounds belong to alcohols, aldehydes, ketones, esters, terpenoids, and sesquiterpenoids. These volatile compounds are classified as alcohols, ethers, or oxides, aldehydes, ketones, esters, amines, phenols, heterocycles, and most notably terpenes and sesquiterpenes.

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Table 9. Structures of Some of the Identified Compounds (Table 8)

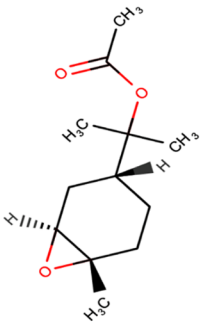
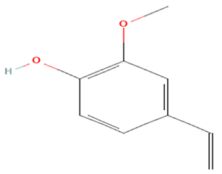
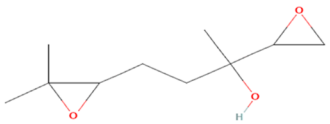
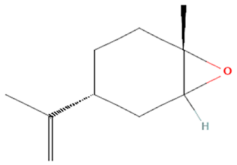
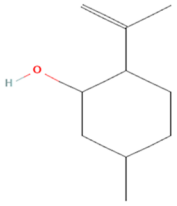
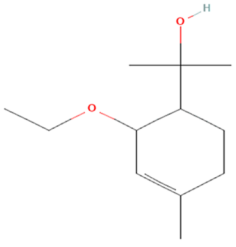
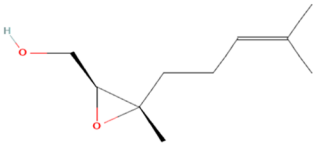
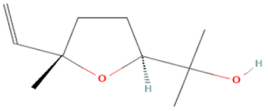
S. No	Name of the compound	Structures
1	Epoxy-alpha- terpinyl acetate	
2	2-Methoxy-4-vinylphenol	
3	Epoxy-linalool oxide	
4	Limonene oxide cis-	
5	dl-Isopulegol	
6	3-Ethoxy-p-menth-1- en-8-ol	
7	Oxiranemethanol, 3-methyl-3-(4-methyl-3-pentenyl)-	
8	Trans-Linalool oxide (furanoid)	

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Table 9. continued

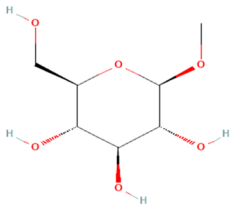
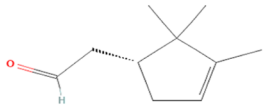
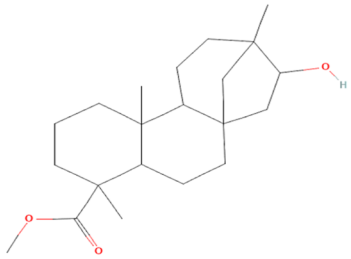
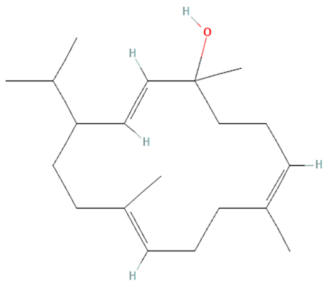
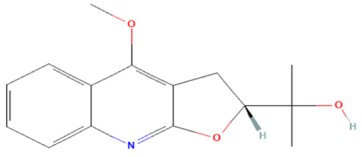
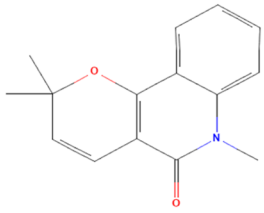
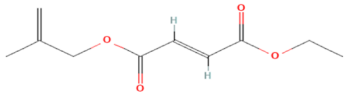
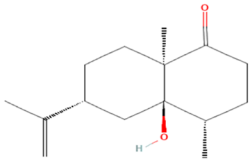
S. No	Name of the compound	Structures
9	Methyl beta-D-Glucopyranoside	
10	Alpha-Campholenal	
11	Methyl dihydroisosteviol	
12	Thunbergol	
13	Platydesmine	
14	2,2,6 Trimethyl 2H,5H,6H pyrano[3.2] quinolin-5-one	
15	Ethyl 2-methylallyl Ester	
16	Corymbolone	

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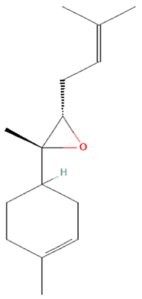
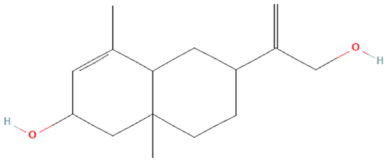
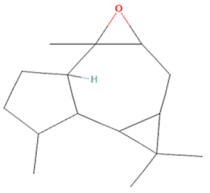
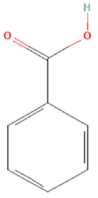
Table 9. continued

S. No	Name of the compound	Structures
17	cis-Z-alpha-Bisabolene epoxide	
18	Bicyclo[4.4.0]dec-2-ene-4-ol	
19	Isoaromadendrene epoxide	
20	Benzoic acid	

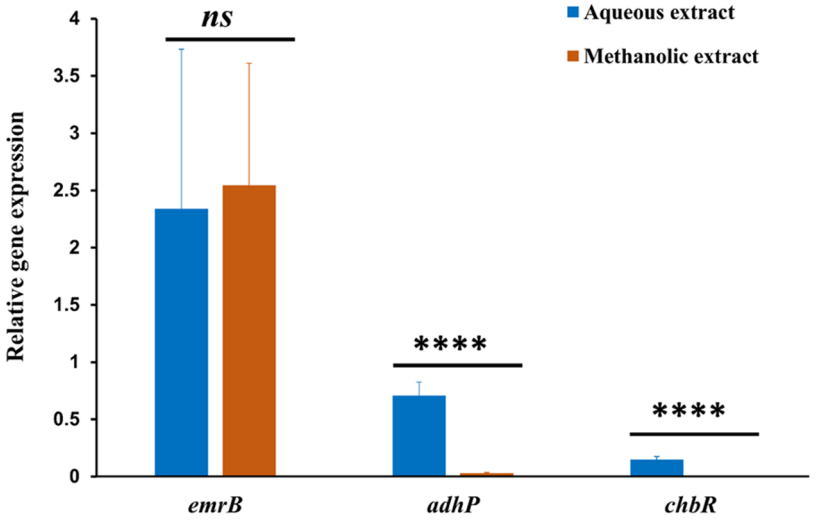


Figure 7. Bar graph showing the relative gene expression of three bacterial strains after treatment with an aqueous and methanolic extract. The relative gene expression values represent the fold change. The Shapiro test followed by two samples *t* test was done to compare the expression between the two extracts. ns indicate that no significant difference was found, while **** indicates *P*-values >0.0001.

The major components present in the both extracts were epoxy-α-terpenyl acetate, 2-methoxy-4-vinylphenol, epoxy-linalool oxide, limonene oxide cis-, DL-isopulegol, 3-ethoxy-*p*-menth-1-en-8-ol, *trans*-linalool oxide (furanoid), α-camphole-nal, α-methyl-α-4-methyl-3-pentenyl oxiranemethanol, (−)-globulol, and methyl dihydroisosteviol. As a result of the presence of these important components, the methanol

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extracts of *O. kilimandscharicum* could have an important therapeutic significance.

5. CONCLUSIONS

The current investigation aimed to identify several phytochemicals and GC-MS characteristics that may be useful for human and animal health. Our results demonstrated that various extracts of *O. kilimandscharicum* contain considerable quantities of phytochemicals that can be potentially used for medicinal purposes. We also demonstrated for the first time the antibacterial effect of the Kenyan cultivars. Additionally, we identified some major compounds that can be useful for *in vivo* and *in vitro* pharmacological screening. Also, methanolic extract was found to be more efficient in antibacterial treatment compared to the water extract. Hence, our study paves the way for future in-depth investigations toward the discovery of efficient biomolecules that could be useful in human and animal health.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05554.

All chemicals and reagents were procured from certified suppliers and were of the highest analytical standard (LEGACY LAB AFRICA LTD.; P.O. Box 50192-00200, Nairobi) and for cell culture, MEM medium, fetal bovine serum (FBS), L-glutamine, antibiotic (penicillin/streptomycin), and resazurin were obtained from (Solarbio China) (PDF)

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Notes

The authors declare no competing financial interest.

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