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Antibacterial properties of some medicinal plant extracts against pathogenic bacteria forming biofilms: Bioactive compounds identification from potential extract and cytotoxicity activity

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Abstract

Medicinal plant extracts are excellent resources of bioactive compounds with perfect antibacterial and antioxidant activity. Phytochemicals are an exciting source of natural pharmaceuticals that are applied instead of synthetic ones and control the growth of multidrug resistant bacteria. About 41.66% of Gram-positive bacteria were detected as biofilm-forming bacteria, while 36.66% were classified as biofilm-forming bacteria belonging to the *Enterobacteriaceae* family. Marigold aquatic extract scored the highest inhibition zone with 7.4 to 13.7%, followed by Moringa with 7.4 to 12.6% against all G^{+ve} bacteria. While the tested *Enterobacteriaceae* G^{-ve} shows higher resistance to the effect of aquatic extracts. The inhibition zone of ethanolic extracts reached 8.4 to 26.3% for Sage, followed by Cinnamon and marigold, which scored 9.5 to 22% and 7.4 to 21%, respectively. The extracts yield percentages ranged from 11.24 to 12.8%. The clear zone diameter recoded by ethanolic Sage extract against G^{+ve} and G^{-ve} in descending order was *Salmonella Typhimurium* ATCC25566> *Listeria monocytogenes* ATCC7646> *Bacillus cereus* ATCC11778> *Staphylococcus aureus* ATCC5638. The bacterial activity of all ethanolic plant extracts achieved the greatest clear zone against tested bacteria, whereas chloroform and methanol recorded the weakest solvents. According to examination using gas chromatography and mass spectrometry, ethanolic Sage extract contains 27 different phytochemical components, with 13-Docosenamide, (Z)- having the highest area with a percentage 14.4%. At doses up to 100 µg/ml, the ethanolic Sage extract showed no cytotoxicity effect to the typical Vero cell line, whereas the IC_{50} value reached 190.67±4.23µg/ml.

Keywords: Antibacterial activities, Cytotoxicity effect, GC/MS analysis, Medicinal plant extract, Solvents extraction.

1. Introduction

Control of spoilage and pathogenic microorganisms that cause food-borne diseases and intoxications are of great interest to scientists as ingredients in human medications [1, 2]. Foodborne diseases are one of the most common causes of diseases and death rates, particularly in countries with low incomes with inadequate sanitary conditions and medical facilities. More than 250 000 deaths related to antimicrobial resistance (AMR) were brought on by many bacteria, including, *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa Streptococcus aureus* and *Staphylococcus pneumonia*. The currently available treatments for these infections have varying degrees of limited effectiveness, adverse effects, and cost-effectiveness [3, 4].

Antibiotic-resistant bacterial infections have significantly risen due to an increase in multidrug-resistant bacteria and the lack of truly effective antimicrobial medicines. The development of new antimicrobial medications and their associated formulations is now more important than ever [5]. According to recent predictions, drug-resistant bacterial infections would result in 10 million mortality per year by 2050 [6]. The yearly pipeline report from the World Health Organization states that the antimicrobial drugs now used in industrial settings are insufficient to fight against the growing danger of antibiotic resistance. Consequently, there

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has been a surge in interest in researching and creating new antibacterial medications [7, 8].

Effective and safe antibacterial agents must be found and developed in order to combat the problem of multidrug-resistant infections. To date, active plant compounds with antibacterial activity have been discovered in order to create new, promising medications. Several antibacterial substances are present in plants and their different parts.

Chemicals from medicinal plants may inhibit bacterial, fungal, viral, and protozoal growth via mechanisms different from those carried out by currently used antimicrobials in situations when were utilized to treat resistant microbial strains [9]. While some of those active chemical compounds lack the efficacy as antibiotics on their own, when used in conjunction with antibiotics, they can aid in overcoming bacterial developed resistance to antibiotics. Some of these active chemical compounds exhibit both antimicrobial resistance-modifying and essential antibacterial activities. Chemically complex substances have a substantial therapeutic potential because they cause few side effects and a lower chance of developing drug resistance than synthetic drugs [10, 11].

The Gram-negative and Gram-positive bacteria are forming extracellular clusters contained by a matrix classified as biofilms. Industrial contamination is mostly caused by biofilms, which exhibit antimicrobial resistance when infections occur. Even though most biofilm communities that occur naturally enclose both G^{+ve} and G^{-ve} bacteria, many biofilm investigations focus on specific Gram type cultures. The Gram-negative bacteria lipopolysaccharides (LPS) and The Gram-positive bacterial cell wall glyco- polymers appear to perform similar roles during initial adhesion [12]. Bacterial cells are acquired resistance by biofilms to host immune systems and environmental stresses. It is possible that a decreased rate of growth rate of bacteria in biofilms, maybe as a result of limited antibiotic penetration into biofilms, the strong efflux pump expression, and expression and exchanging of resistance genes amongst bacteria inside a biofilm are all contributing factors to the increasing of antibiotic resistance pattern exhibited by biofilm- forming bacteria as compared to bacteria that do not form biofilm [13, 14]. The current study's main aim is to i) assess the antibacterial activity of a number of medicinal plant extracts towards bacteria that form biofilms, both Gram-positive and Gram-negative. ii) Identifying the most efficient organic solvent to prepare plant extracts. iii) Applying gas chromatography and mass spectrometry(GC-MS) to determine the phytocomponents in the selected ethanol plant extracts and evaluate their cytotoxic properties.

2. Material and methods

2.1. Bacterial pathogens and inoculum preparation

The pathogenic bacterial strains and isolates used in this current investigation originated from the Department of Agricultural Microbiology, Faculty of Agriculture, Ain-shams University and Microbiological Resource Centers (MIRCEN), Cairo, Egypt. Only 12 G^{+ve} (*Staphylococcus aureus* ATCC6536, *S. aureus* ATCC25923, *S. aureus* ATCC6538, *S. aureus* ATCC43300, *Staphylococcus* sp. codes from 1 to 3, *Listeria monocytogenes* ATCC19116, *L. monocytogenes* ATCC7646, *Listeria* sp., *Bacillus cereus* ATCC11778, and *Enterococcus faecalis* 20247_ CHB) and 30 G^{-ve} *Escherichia coli* ATCC8739, *E. coli* O157H7, *E. coli* codes from 1 to 3 , *Enterobacter cloacae* MB11506_1 CHB1, *Ent. cloacae* MB11506_1 CHB4, *Ent. cloacae* MB11506_1 CHB2, *Ent. cloacae* DSM 3264 BRB, *Ent. kobei* DSM 13645T DSM1, *Ent. asburiae* DSM 17506T, *Klebsiella pneumonia* DSM 16358T, *K. pneumonia* DSM 30104T, *K. pneumonia* ATCC 00607, *Klebsiella* sp. codes from 1 to 3, *Pseudomonas aeruginosa* ATCC 27853 THL 2, *Ps. aeruginosa* ATCC 27853, *Pseudomonas* sp. codes from 2 to 7, *S. typhi* ATCC 25566, *Salmonella* sp., *Citrobacter freundii* 22054_1 CHB1, *Acinetobacter* sp., and *Shigella* sp.) bacterial strains were investigated.

To prepare standard inoculum, after 24 h of incubation, 4 to 5 single colonies were grabbed from the bacterial culture of examined pathogenic bacteria and subcultured into 4 ml Müller-Hinton broth medium. After that, the inoculated tubes were incubated at 37°C until the turbidity of 0.5 MacFarland standard was reached [15]. The prepared inoculum was standardized using a spectrophotometer (APEL, Japan) set to detect optical density at 625 nm, which ranged from 0.08 to 0.12. The standardized inoculum of tested bacteria was 2×10^7 CFU/ml.

2.2. Medicinal plant sample collection

Eleven different plant materials were collected from the botanical farm in the Faculty of Agriculture, Ain Shams University, Cairo, Egypt as seen in **Table (1).** The obtained plant material was cleaned with tap water to eliminate particle debris on the surface then allowed to air dry for seven days at room temperature (28±2°C). The dried pieces were milled (Moulinex grinder, France) and sieved to

produce a fine powder. Dried plant material was kept at −20 °C until usage.

Table 1. The medicinal plant's scientific name and the part used for the study

Scientific name	Common name	The used part of the plant
Calendula officinalis	Marigold	Flowers
Cinnamomum veru	Cinnamon	Bark
Curcuma longa	Turmeric	Rhizomes
Matricaria chamomilla	Chamomile	Flowers
Moringa oleifera	Moringa	Leaves
Nigella sativa	Black seeds	Seeds
Psidium guajava	Guava	Leaves
Rosmarinus officinalis	Rosemary	Leaves
Salvadora persica	Miswak	Sticks
Salvia officinalis	Sage	Leaves
Syzygium aromaticum	Clove	Flowers

2.3. Biofilm formation assay

2.3.1. Tube assay

The tube staining approach was used to examine the biofilm's qualitative investigation [16]. The surfaces were washed with acetone, immersed in a detergent (soap) for 1h, carefully washed out with distilled water, and dried for 1h at 160ºC [17]. The tryptic soy broth (TSB), containing 5% sucrose, was inoculated with pathogenic bacterial inoculum (100 µl) and then incubated for 24 hours at 37°C. The inoculated tubes were poured with Phosphate buffer saline (PBS) with pH 7.3 contains 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, and 1.8 mM KH2PO4 to eliminate the planktonic bacterial cells, dried, and then stained with 0.1% crystal violet. Tubes were washed in deionized water to get rid of the remaining stain. The apparent film on the tube wall and bottom acted as evidence that a biofilm had formed. The liquid contact did not appear to be a sign that a biofilm was forming [18].

2.3.2. Modified Congo red agar method (MCRA)

The constituents of the medium are modified Congo red agar according to, [19] which add of (g/l) of blood base agar; 40, Congo red dye; 0.4 and glucose; 10. A concentrated aqueous solution of Congo red stain was made, and for 15 min, it was autoclave (HumanLab, Korea) sterilized at 121°C. Then, it was mixed with the sterilized blood agar base and glucose at 55°C and poured into petri dishes. Tested microorganisms were streaked on the plates and then incubated for (GENLAB, England) 24-48 h at 37°C. Biofilm formation was characterized by crystal-like dry black colonies, and the non-biofilm forming bacterial colonies appear as pink color.

2.4. Preparation of medicinal plant extracts

2.4.1. Aqueous extracts

A 100 ml of sterile, distilled water was poured into a beaker, including 20 g of plant material as powder. The mixture had been heated for three hours at 60°C, continuously shaking in a water bath (Kottermann, Germany). After that, the final extracts were filtered with a double layer of cloth, with Whatman filter paper No. 1 (Double rings filter paper 102, 11cm qualitative), then put in an oven at 40ºC to concentrate it. The crude extract was dissolved in 10 ml of sterile distilled water to create a stock solution and kept at 4°C until needed [20].

2.4.2. Alcoholic and other solvents extracts

Twenty-five grams of each dried powdered plant were mixed separately with 200 ml of 4 organic solvents, including absolute ethanol, acetone, ethyl acetate, chloroform, and methanol**)** PIOCHEM laboratory chemicals, Giza, Egypt) in a continuous shaker (Shin Saeng, Korea) at 40ºC for 48 h. The resulting extract was then passed through filter paper No. 1 and then put in an oven (Heraeus, Germany) at 40ºC to get it concentrated, as the method described by [21]. To prepare the suspension, the extract was allowed to dissolve in 50% dimethyl sulfoxide (DMSO) at pH 7.4 and then diluted with sterile distilled water for usage [22]. The yield of plant extracts was calculated according to [23] as follows in Equation (1):

Plant extract yield (%) = (Weight of extract / Weight of dried plant materials) x 100. **Eq. 1**

2.5. Assessment of medicinal plant extracts antibacterial activity

With this procedure, plates of nutrient agar medium were inoculated with 100 µl of 18–24 hours tested bacterial standard inoculum (containing 2×10^7 CFU/ ml 0.5 McFarland standard). Discs of Whatman No. 1 filter paper (with 5 mm diameter) were autoclaved in a glass petri dish for 20 minutes at 121°C and then put onto the medium with sterilized forceps, as well as 10 µl of each plant extract was applied on the discs. Dimethyl sulfoxide (50% DMSO) was additionally used to be a negative control. The plates were placed into incubator for 24 h at 37°C. The following day, inhibition zones surrounding the discs in each plate were measured in millimeters [24]. The percentage of inhibition rate was performed, ratio of the inhibition zone and diameter of colony in dishes without plant extract.

2.6. Gas Chromatography and Mass spectroscopy analysis (GC/MS) analysis of the most effective extract

At the Centre for Drug Discovery Research and Development Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, the most effective extract was analyzed using GC/MS (Shimadzu GCMS-QP2020, Tokyo, Japan). Restek, USA, 30 m x 0.25 mm i.d. x 0.25 m film thickness Rtx-1MS fused bonded column. Ionization mode: 200°C ion source; 70 eV ionization voltage. Program for controlling temperature: 45°C for 2 min, followed by 5 min of stable temperature at 300°C (isothermal). 250°C is the injector's temperature helium is the carrier gas, with a flow rate of 1.41 ml/min. (1% v/v) for diluted samples. Split mode was used to inject the sample (1:15 split ratio). Wiley & Nist Mass Spectral Data Base Library was searched in.

2.7. Cytotoxicity of the most effective extract on Vero cell line

The cytotoxic impact of the most effective extract on Vero cell line (a normal kidney CCL-81) was investigated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) study reported by [25] technique. This experiment was carried out at the Science Way for Scientific Researches and Consultations Company in Cairo, Egypt. The 96-well tissue culture plate created a complete monolayer sheet after 24 hours of incubation at 37°C with 100 µl/well of standard cellular viability inoculation (1 x 10^5 cells/ml). After the growing sheet of cells had formed in 96-well microtiter plates, the growth medium was withdrawn, and the cell monolayer was repeatedly washed with wash medium. The most effective extract was diluted twice in the Roswell Park Memorial Institute (RPMI) medium (maintenance medium), which contains 2% serum. The other wells received 0.1 ml of each dilution for testing, while three wells served as controls and just received maintenance medium. At 37°C, the plate was incubated before being inspected. The MTT solution was prepared using (5 mg/ml in phosphate-buffered saline solution) (BIO BASIC CANADA INC.). Twenty microliters of MTT solution were added to each well, and the media was subsequently combined with the solution by shaking the wells for five minutes at 150 rpm. The MTT was then metabolized by the cells for 4 hours at 37°C and 5% CO_2. Formazan (an MTT metabolic product) should be redissolved in 200 µl of DMSO and shaken at 150 rpm on a shaking table for five minutes to combine the formazan and solvent. The optical density, the cell amount, was calculated using the absorbance for each well at 560 nm in a microplate reader (FLUOstar Omega). Morphological cells from a cytotoxicity study were examined for any physical signs of toxicity after being treated with the most effective extract. GraphPad Prism version 5 was used to determine the "half-maximal (50%) inhibitory concentration (IC_{50})". The changes include partial or complete monolayer loss, rounding, shrinkage, or cell granulation.

2.8. Statistical analysis

In accordance with Duncan's multiple range tests at 0.05 [26], data was analyzed using IBM® SPSS ® statistics software version 19. A one-way analysis of variance (ANOVA) was used to evaluate the significance of the variance among groups.

3. Results and discussion

3.1. Pattern of pathogenic bacteria for biofilm formation

A total of 42 pathogenic bacteria (12 G^{+ve} and 30 G^{-ve} bacteria) were collected, and their capability to form biofilm was evaluated using tube assay and MCRA methods. Results in **Figure (1a)** showed that only 5 G^{+ve} bacteria (41.67%) and 11 G^{-ve} tested bacteria (36.67%) were able to form a biofilm which was visible as a pattern on a glass tube and crystal-like dry black colonies on modified Congo red agar, respectively **(Figure 1b)**. The biofilm-forming G^{-ve} bacteria were found belonging to the *Enterobacteriaceae* family. As stated by [12], that the pattern of biofilm formation was similar between both G^{+ve} and G^{-ve} bacteria despite the variances in their genomes, physiology, and the envelopes of the outer cell. In addition, both bacterial types have a specific component in their cell wall enhanced biofilm formation as per LPS in G^{-ve} bacteria, a thick peptide-glucan layer and teichoic acid in G^{+ve} bacteria [27, 28]. Generally, all Gram-positive and Gram negative bacteria that have flagella move via motility and the surface condition by emission of polysaccharides to support the attachment of the bacterial cells and create biofilm [29].

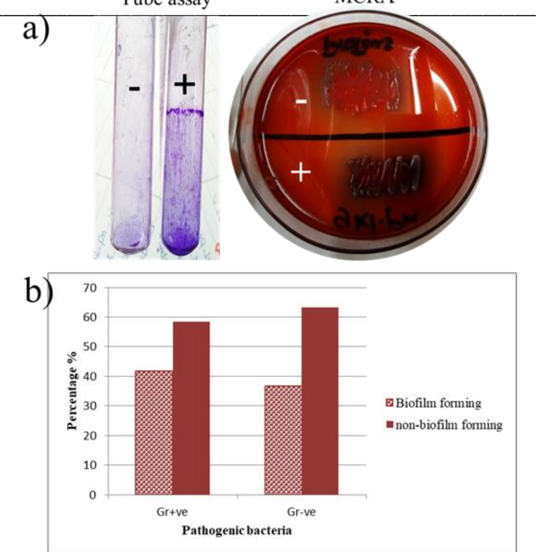


Fig.1. Pattern of Gr^{+ve} and Gr^{-ve} bacteria for biofilm formation (a) and biofilm detection by tube assay and MCRA method (b).

3.2. Antibacterial evaluation of aquatic plant extracts

The antibacterial effluence of 11 aquatic extracts as antibacterial agents varied in their activity against G^{+ve} or G^{-ve} biofilm forming bacteria **(Tables 2& 3)**. All aquatic extracts inhibit all G^{+ve} tested bacteria except Black seeds and Cinnamon extracts, affecting only the growth of *S. aureus* strains for the former extract and *S. aureus* ATCC6536, *S. aureus* ATCC5638 & *L. monocytogenes* ATCC7646 for the latter, as scored the lowes inhibition zone ranged from 7-9 mm (with percentage 7.4 - 9.5%) by the former extract which recorded the lowest extract yield being 3.9%. Also, it could be noticed that *B. cereus* ATCC11778 was more resistant to tested extracts, followed by *S. aureus* ATCC5923 and *L. monocytogenes* ATCC7646, which recorded mean values of 6.91, 7.36, and 7.82 mm, respectively, whereas *S. aureus* ATCC5638 was more sensitive to these extracts (10.4 mm of the mean value). On the other hand, a highly significant effect on all tested G^{+ve} strains was aqueous Clove extract by 10 mm mean inhibition zone followed by Moringa, Marigold, or Sage. Marigold scored higher in the inhibition zone ranged from 7 to 13 mm (with percentage 7.4 to 13.7%), followed by Moringa in the range of 7-12 mm (with percentage 7.4 to 12.6%) against G^{+ve} bacteria and achieved a moderate yield of 8.25% and 5.1%, respectively. The highest Marigold clear zone 13 mm (13.7%) was introduced against *S. aureus* ATCC5638. The *Enterobacteriaceae* G^{-ve} tested bacteria show higher resistant to the effect of aquatic extracts, only Sage, Moringa, Turmeric, and Miswak almost inhibited all tested bacteria as well as inhibition zone ranged from 7 to 10 mm (with percentage 7.4 to 10.5%), also the highest yield of 14.75% was obtained by Sage. The lowest value of mean inhibition zone diameter attained by all tested aqueous extracts was obtained by *En. Cloacae* MB11506_1 CHB1 followed by *Pseudomonas* sp. 7 and *E. coli* O157H7 being 6.82, 7.27 and 7.6 mm, respectively. The R^2 for the inhibition zone of G^{+ve} and G^{-ve} bacteria was 0.98. The final results of the antibacterial effectiveness of the tested plant extracts are consistent with the findings of [30, 31] who stated that the lipopolysaccharides and phospholipids found within the outer membrane of the Gram-negative bacteria raise the membrane's negative charge and allowed for stabilization of its structure against antibacterial agents.

Numerous phytochemicals found in medicinal plants, including phenolic acids, tannins, terpenoids, flavonoids, and alkaloids, have a variety of active mechanisms, including various antibacterial actions. Thus, the -OH group(s) linked to the phenol ring is responsible for the antibacterial activity of these plants' secondary metabolites [32]**.** Moreover, the mode of action is related to the plant varieties. Some relate to the connection between the proteins or enzymes and the bacterial cell membrane, while others affect the disintegration of the phospholipid structure and the reduced gradient of pH within the membrane. Some others degrade the cell wall or destroy the cytoplasmic and protein membranes, allowing the leakage of the cell content [33, 34]. Other investigations reported that roselle water has high antibacterial activity against *B. cereus* being 17 mm; in addition, *B. cereus* stated extra sensitivity of clove water extract with a low MIC of 0.32% [34].

Table 2
Antibacterial activity inhibition zones of the aqueous medicinal plant extracts against Gram-positive bacteria.

Plant extracts	Conc. (mg/ml)	Yield (%)	*B. cereus* ATCC11778		*L. monocytogenes* ATCC7646		*S. aureus* ATCC 5923		*S. aureus* ATCC 6536		*S. aureus* ATCC5638		Mean
			IZ	(%)	IZ	(%)	IZ	(%)	IZ	(%)	IZ	(%)	
Black seeds	113	3.90	-	-	-	-	8	8.4	7	7.4	9	9.5	4.8^{f}
Chamomile	259	13.00	10	10.5	9	9.5	7	7.4	10	10.5	9	9.5	9.0^{c}
Cinnamon	113	5.65	-	-	8	8.4	-	-	12	12.6	11	11.5	6.2^{e}
Clove	138	6.90	10	10.5	10	10.5	9	9.5	10	10.5	11	11.5	10^{a}
Guava	157	5.90	8	8.4	8	8.4	8	8.4	9	9.5	9	9.5	8.2^{d}
Marigold	165	8.25	7	7.4	9	9.5	9	9.5	9	9.5	13	13.7	9.4^{b}
Miswak	228	8.55	9	9.5	8	8.4	7	7.4	9	9.5	11	11.5	8.8^{c}
Moringa	102	5.10	7	7.4	9	9.5	9	9.5	12	12.6	11	11.5	9.6^{b}
Rosemary	261	13.05	9	9.5	8	8.4	8	8.4	9	9.5	10	10.5	9,0^{c}
Sage	295	14.75	10	10.5	9	9.5	8	8.4	10	10.5	10	10.5	9.4^{b}
Turmeric	117	6.65	7	7.4	8	8.4	8	8.4	8	8.4	10	10.5	8.2^{d}
Mean			6.91^{e}		7.82^{c}		7.36^{d}		9.55^{b}		10.4^{a}		

Statistical analysis of variance (ANOVA)

Source	df	*F*-value	*p*-value	R²
Corrected Model	54	96.6	0.0001	0.98
Intercept	1	50222.1		
Isolates	4	313.3		
Treatment	10	158.9		

(-) = no effect, **IZ**= Inhibition zone diameter (mm), **Conc.**= concentraction, **df**= degree of freedom, ***F***= corresponding level of significance, ***p***= corresponding level of significance, **R²**= Determination coefficient. [a, b] Values having small letters in the same column and row with different superscripts demonstrate a significant difference, and values with the same letter do not significantly differ from one other, according to Duncan's at $p \leq 0.05$.

3.3. Antibacterial effluence evaluation of ethanolic plant extracts

All tested G^{+ve} bacteria introduce a clear zone of growth with all ethanolic plant extracts except black seed affecting only *L. monocytogenes* ATCC7646, *S. aureus* ATCC6536, and *B. cereus* ATCC7646 with 9 mm inhibition zone (with percentage 9.5%) and produce the lower yield being 8.8%. The mean value of the inhibition zone of the ethanolic extracts was improved to reach (19.80 mm) by Sage, followed by Cinnamon (14.60 mm) and Marigold (13.60 mm), while extracts yield ranged from 11.24 to 12.80%. Whereas the lowest mean value of the inhibition zone for all G^{+ve} bacteria tested, 5.4 mm was attained by Black seeds ethanolic extract, which has no effect on *S. aureus* ATCC5923 and *S. aureus* ATCC5638 growth, as seen in **Table (4).** With respect to G^{-ve} bacteria reported the same trend of G^{+ve}, the ethanolic extracts are in descending order as follow: Sage> Cinnamon> Marigold with inhibition zone ranged from 8 to 26 (with percentage 8.4 to 27.3%), 10 to 17 (with percentage 10.5 to 18%), and 7 to 16 mm (with percentage 7.4 to 16.8%), respectively **(Table 5).** The clear zone diameter recorded by Sage against G^{+ve} and G^{-ve} in descending order was *S. Typhimurium* 22 ATCC25566> *L. monocytogenes* ATCC7646> *B. cereus* ATCC11778> *S. aureus* ATCC5638. The mean of the inhibition zone varied significantly according to the tested bacteria and ranked in ascending order as follows: *B. cereus* ATCC11778< *S. aureus* ATCC5638< *S. Typhimurium* ATCC25566< *L. monocytogenes* ATCC7646. Sage and Cinnamon show significant effects against both G^{+ve} and G^{-ve} bacteria with a high mean of 19.8 & 14.60 mm and 13.45 &11.55 mm, respectively, which clearly show that the ethanolic extracts have greater effectiveness against G^{+ve} bacteria.

As stated by [35] ethanolic extracts of *Cymbopogon citratus* and *Azadirachta indica* leaves had significantly higher content of flavonoids and phenolic than the aqueous extracts with respect to phytochemistry and antibacterial activity. There were more elevated amounts of caffeic acid and sinapic acid with concentrations of 6.74 mg/g and 1.43 mg/g, respectively, in *C. citratus* ethanolic extract. The *C. citratus* pure extract has the strongest antibacterial activity, achieving a greater effect than the common antibiotic (ciprofloxacin). In comparison to *E. coli*, both individually and in combination, the plant extracts were significantly more efficient

against *S. aureus*. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the two bacterial species were decreased in the ethanolic extract of ginger compared with the aquatic extract. The ethanolic extract of ginger was 1.6-Fold and 2-Fold more efficient than the aqueous extract at inhibiting the growth of *S. pneumoniae* and *P. aeruginosa*, respectively, with a 2g/ml concentration [36].

Table 3
Inhibition Zones of the aqueous medicinal plant extracts against Gram-negative bacteria.

Plant extracts	Conc. (mg/ml)	Yield (%)	Pathogenic bacteria																						Mean
			E. coli ATCC 8739		*E. coli* O157H7		*E. coli* 2		*Ent. cloacae* MB11506_1 CHB1		*Ent.* cloacae MB11506_1 CHB4		*K. pneumonia* DSM 16358T		*K. pneumonia* DSM 30104T		*K. pneumonia* ATCC 00607		*Ps. aeruginosa* ATCC 27853 THL 2		*Pseudomonas* sp. 7		*S. typhi* ATCC 25566		
			IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	
Black seeds	113	3.90	8	8.4	7	7.4	8	8.4	-	-	9	9.5	-	-	9	9.5	8	8.4	-	-	10	10.5	9	9.5	6.18^{f}
Chamomile	259	13.00	10	10.5	9	9.5	8	8.4	8	8.4	10	10.5	9	9.5	9	9.5	9	9.5	10	10.5	9	9.5	8	8.4	9.00^{b}
Cinnamon	113	5.65	8	8.4	7	7.4	8	8.4	9	9.5	8	8.4	9	9.5	8	8.4	8	8.4	10	10.5	7	7.4	-	-	7.45^{e}
Clove	138	6.90	9	9.5	10	10.5	9	9.5	8	8.4	11	11.5	9	9.5	9	9.5	10	10.5	10	10.5	-	-	9	9.5	8.55^{c}
Guava	157	5.90	7	7.4	-	-	7	7.4	-	-	8	8.4	9	9.5	-	-	8	8.4	9	9.5	10	10.5	7	7.4	5.91^{g}
Marigold	165	8.25	9	9.5	8	8.4	8	8.4	8	8.4	-	-	8	8.4	7	7.4	9	9.5	9	9.5	9	9.5	9	9.5	7.64^{e}
Miswak	228	8.55	8	8.4	8	8.4	8	8.4	8	8.4	10	10.5	8	8.4	9	9.5	8	8.4	8	8.4	9	9.5	9	9.5	8.45^{c}
Moringa	102	5.10	9	9.5	8	8.4	9	9.5	8	8.4	10	10.5	10	10.5	9	9.5	9	9.5	9	9.5	9	9.5	10	10.5	9.09^{b}
Rosemary	261	13.05	8	8.4	9	9.5	7	7.4	9	9.5	8	8.4	11	11.5	8	8.4	7	7.4	8	8.4	-	-	9	9.5	7.64^{e}
Sage	295	14.75	8	8.4	10	10.5	9	9.5	10	10.5	9	9.5	10	10.5	10	10.5	10	10.5	10	10.5	10	10.5	8	8.4	9.45^{a}
Turmeric	117	6.65	8	8.4	8	8.4	7	7.4	7	7.4	8	8.4	7	7.4	8	8.4	8	8.4	9	9.5	7	7.4	10	10.5	7.91^{d}
Mean			8.36^{ab}		7.60^{e}		8.00^{cd}		6.82^{g}		8.27^{b}		8.18^{bc}		7.82^{de}		8.55^{a}		8.36^{ab}		7.27^{f}		8.00^{cd}		

Statistical analysis of variance (ANOVA)

Source	df	F-value	p-value	R^2
Corrected Model	120	86.3	.0001	0.98
Intercept	1	99632.4		
Isolates	10	39.0		
Treatment	10	187.2		

(-) = no effect, **IZ**= Inhibition zone diameter (mm), **Conc.=** concentraction, **df**= degree of freedom, ***F***= corresponding level of significance, ***p***= corresponding level of significance, **R²**= Determination coefficient. $^{a, b}$ Values having small letters in the same column and row with different superscripts demonstrate a significant difference, and values with the same letter do not significantly differ from one other, according to Duncan's at $p \leq 0.05$.

Table 4
Inhibition zone diameters of the ethanolic medicinal plant extracts against Gram-positive bacteria.

Plant extracts	Conc. (mg/ml)	Yield (%)	Pathogenic bacteria										Mean
			B. cereus ATCC11778		*L. monocytogenes* ATCC7646		*S. aureus* ATCC 5923		*S. aureus* ATCC 6536		*S. aureus* ATCC5638		
			IZ	(%)	IZ	(%)	IZ	(%)	IZ	(%)	IZ	(%)	
Black seeds	259	8.80	9	9.5	9	9.5	-	-	9	9.5	-	-	5.4^{i}
Chamomile	190	7.60	14	14.7	18	19	9	9.5	10	10.5	16	16.8	13.4^{c}
Cinnamon	200	11.24	12	12.6	21	22	10	10.5	9	9.5	21	22	14.6^{b}
Clove	300	11.00	11	11.5	13	13.7	8	8.4	11	11.5	16	16.8	11.8^{d}
Guava	150	6.00	10	10.5	14	14.7	8	8.4	10	10.5	9	9.5	10.2^{g}
Marigold	320	12.80	12	12.6	20	21	10	10.5	9	9.5	17	18	13.6^{c}
Miswak	156	3.12	10	10.5	14	14.7	10	10.5	7	7.4	11	11.5	10.4^{fg}
Moringa	241	7.24	10	10.5	17	18	9	9.5	7	7.4	10	10.5	10.6^{f}
Rosemary	440	17.60	10	10.5	13	13.7	11	11.5	10	10.5	12	12.6	11.2^{e}
Sage	150	12.00	24	25.3	25	26.3	14	14.7	14	14.7	22	22	19.8^{a}
Turmeric	92	3.68	10	10.5	13	13.7	9	9.5	7	7.4	10	10.5	9.8^{h}
Mean			12.00^{c}		16.09^{a}		8.91^{e}		9.36^{d}		13.09^{b}		

Statistical analysis of variance (ANOVA)

Source	df	F-value	p-value	R^2
Corrected Model	54	302.4	.0001	0.99

Intercept	1	96841.4
Isolates	4	1177.0
Treatment	10	802.1

(-) = no effect**, IZ=** Inhibition zone diameter (mm), **Conc.=** concentraction, **df=** degree of freedom, ***F=*** corresponding level of significance, ***p=*** corresponding level of significance, **R^2=** Determination coefficient. [a, b] Values having small letters in the same column and row with different superscripts demonstrate a significant difference, and values that have the same letter do not significantly differ from one other, according to Duncan's at $p \leq 0.05$.

Table 5

Inhibition zone diameters of the ethanolic medicinal plant extracts against Gram-negative bacteria.

			pathogenic bacteria																						
Plant extracts	Conc. (mg/ml)	Yield (%)	*E. coli* ATCC 8739		*E. coli* O157H7		*E. coli* 2		*Ent. cloacae* MB11506_1 CHB1		*Ent.* cloacae MB11506_1 CHB4		*K. pneumonia* DSM 16358T		*K. pneumonia* DSM 30104T		*K. pneumonia* ATCC 00607		*Ps. aeruginosa* ATCC 27853 THL 2		*Pseudomonas* sp. 7		*S. typhi* ATCC 25566		Mean
			IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	IZ	%	
Black seeds	259	8.8	8	8.4	9	9.5	8	8.4	-	-	9	9.5	9	9.5	10	10.5	8	8.4	-	-	10	10.5	9	9.5	7.27^{h}
Chamomile	190	7.6	9	9.5	11	11.5	9	9.5	8	8.4	10	10.5	16	16.8	9	9.5	10	10.5	10	10.5	9	9.5	15	15.8	10.55^{d}
Cinnamon	200	11.24	12	12.6	10	10.5	10	10.5	10	10.5	14	14.7	16	16.8	10	10.5	11	11.5	-	-	17	18	17	18	11.55^{b}
Clove	300	11	9	9.5	11	11.5	11	11.5	12	12.6	11	11.5	12	12.6	9	9.5	12	12.6	11	11.5	10	10.5	12	12.6	10.91^{c}
Guava	150	6	8	8.4	10	10.5	10	10.5	8	8.4	9	9.5	10	10.5	8	8.4	9	9.5	9	9.5	-	-	9	9.5	8.18^{g}
Marigold	320	12.8	-	-	9	9.5	9	9.5	9	9.5	10	10.5	16	16.8	11	11.5	10	10.5	8	8.4	7	7.4	16	16.8	9.55^{e}
Miswak	156	3.12	9	9.5	9	9.5	8	8.4	12	12.6	8	8.4	10	10.5	8	8.4	8	8.4	-	-	9	9.5	12	12.6	8.45^{f}
Moringa	241	7.24	8	8.4	9	9.5	7	7.4	-	-	-	-	10	10.5	8	8.4	8	8.4	8	8.4	8	8.4	12	12.6	7.09^{h}
Rosemary	440	17.6	9	9.5	11	11.5	10	10.5	13	13.7	-	-	12	12.6	9	9.5	10	10.5	10	10.5	-	-	11	11.5	8.64^{f}
Sage	150	12	8	8.4	14	14.7	15	15.8	12	12.6	8	8.4	22	23.1	13	13.7	13	13.7	9	9.5	8	8.4	26	27.3	13.45^{a}
Turmeric	92	3.68	8	8.4	-	-	-	-	-	-	10	10.5	11	11.5	8	8.4	8	8.4	8	8.4	9	9.5	12	12.6	6.73^{i}
Mean			8^{f}		9.4^{d}		8.82^{e}		7.64^{g}		8.09^{f}		13.09^{b}		9.36^{d}		9.37^{c}		6.64^{h}		7.91^{f}		13.73^{a}		

Statistical analysis of variance (ANOVA)

Source	df	*F*-value	*p*-value	R^2
Corrected Model	120	244.9	.0001	0.988
Intercept	1	140875		
Isolates	10	729.7		
Treatment	10	658.4		

(-) = no effect**, IZ=** Inhibition zone diameter (mm), **Conc.=** concentraction **, df=** degree of freedom, ***F=*** corresponding level of significance, ***p=*** corresponding level of significance, **R^2=** Determination coefficient.[a, b] Values having small letters in the same column and row with different superscripts demonstrate a significant difference, and values with the same letter do not significantly differ from one other, according to Duncan's at $p \leq 0.05$.

3.4. Detection of the most efficient solvent for extraction

The conformation of every plant extract diverges according to the plant and the extraction procedure as well as the solvent used. So, thus, 4 organic solvents, including ethanol, acetone, ethyl acetate, chloroform, and methanol, affected the antibacterial activity of Sage, Cinnamon, Chamomile, and Marigold, as seen in Table (6). All ethanolic extract recorded the utmost clear zone and yield. Sage ethanolic extracts show the strongest antibacterial, clear zone diameter ranging from 22 to 26 mm (with percentage 23.1 to 27.3%). Also, the diameter of the clear zone enlarged by about 8, 9, and 11 mm (with percentage 8.4, 9.5 and 11.5%) against *S. aureus* ATCC5638, *S. Typhimurium* ATCC25566, and *B. cereus* ATCC11778, respectively, compared with that obtained by chloroform. In addition, methanol and chloroform were inefficient with Cinnamon and reduced the inhibition zone by about 38.1% (compared to ethanol) against *S. aureus* ATCC 5638. The reduction in the inhibition zone scored 40% with marigold chloroform extract against *L. monocytogenes* ATCC11778. The lowest percentage of yield extract of all tested plants was obtained by chloroform followed by acetone, ranged from 0.32 to 1.2% and 0.4 to 2.0%, respectively.

Moreover, all ethanolic extracts have a significant effect on the antibacterial activity (p< 0.05) and scored the highest mean of inhibition zone being 24.25, 17.75, 16.25, and 15.75 mm for Sage, Cinnamon, Marigold, and Chamomile, respectively. This may associate with the antibacterial activity of bioactive chemical compounds represented in ethanolic plant extract, which is highly efficient against every examined microbial species [37]. Furthermore, the mean of ethyl acetate plant extracts scored the second rank amoung all tested medicinal plants means with a range from 11.5 to 19.5.

Moreover, the variation in the effectiveness of the extracts against tested strains may attributed to their phytochemical composition, which includes tannins, flavonoids, saponins, steroids, alkaloids, and triterpenes [38]. In this respect, [39] had been noticed that the ethanolic extract of *Pterolobium stellatum* leaves had a more potent antibacterial effect on some pathogens, particularly *Streptococcus pyogenes, Shigella* sp., *Salmonella* sp., *Escherichia coli*, *Pseudomonas* sp. and *S. aureus*. All bacteria showed to have statistically significant (p<0.05)

growth inhibition in an ethanol extract, with *Shigella* spp. showing the maximum inhibition (21.33±1.52 mm), whereas chloroform extract had a less significant inhibition ($p<0.05$). As reported by [40], with the potential exception of sage extract, plant extracts attained by utilizing an ecologically friendly solvent had no toxicity, such as an aqueous ethanolic solution contains a higher content of phenolic compounds than an aqueous methanolic solution. However, there were no statistically significant variations in the bactericidal activity of the residual ethanolic and methanolic extracts. As a result, aqueous ethanolic plant extracts rather than aqueous methanolic extracts are suggested as natural antibacterial agents.

Some researchers noticed that the aquatic extracts of various plants typically give considerably greater yields when compared with ethanolic extracts of the same plants due to the stronger polarity of water and applying a high temperature for 30 min for the extraction period [41, 42].

Table 6
The inhibition zone diameters of plant extracts using different organic solvents against the most suspected pathogenic bacteria

Plant extracts	Solvent	Conc. (mg/ml)	Yield (%)	*S. aureus* ATCC5638		*B. cereus* ATCC11778		*L. monocytogenes* ATCC7646		*S. Typhimurium* ATCC25566		
				IZ	**(%)**	**IZ**	**(%)**	**IZ**	**(%)**	**IZ**	**(%)**	**Mean**
Chamomile	Ethanol	190	7.6	16	16.8	14	14.7	18	19	15	15.8	15.75^{a}
	Acetone	25	0.4	12	12.6	11	11.5	9	9.5	13	13.7	11.25^{d}
	Ethyl acetate	75	1.2	14	14.7	12	12.6	14	14.7	14	14.7	13.50^{b}
	Chloroform	75	1.2	13	13.7	11	11.5	12	12.6	11	11.5	11.75^{c}
	Methanol	133	3.2	15	15.8	10	10.5	8	8.4	13	13.7	11.50^{cd}
Cinnamon	Ethanol	200	11.24	21	22.1	12	12.6	21	22.1	17	18	17.75^{a}
	Acetone	63	2	14	14.7	11	11.5	20	21	15	15.8	15.00^{b}
	Ethyl acetate	125	2	14	14.7	10	10.5	19	20	16	16.8	14.75^{b}
	Chloroform	50	0.8	13	13.7	10	10.5	20	21	14	14.7	14.25^{c}
	Methanol	133	3.2	13	13.7	9	9.5	20	21	13	13.7	13.78^{d}
Marigold	Ethanol	320	12.8	17	18	12	12.6	20	21	16	16.8	16.25^{a}
	Acetone	75	1.2	11	11.5	10	10.5	12	12.6	11	11.5	11.00^{c}
	Ethyl acetate	300	4.8	11	11.5	10	10.5	14	14.7	11	11.5	11.50^{b}
	Chloroform	35	0.56	11	11.5	9	9.5	12	12.6	11	11.5	10.75^{c}
	Methanol	183	4.4	15	15.8	9	9.5	13	13.7	9	9.5	11.50^{b}
Sage	Ethanol	150	12	22	23.1	24	25.3	25	26.3	26	27.3	24.25^{a}
	Acetone	33	0.8	17	18	19	20	15	15.8	19	20	17.50^{d}
	Ethyl acetate	83	2	19	20	20	21	19	20	20	21	19.50^{b}
	Chloroform	20	0.32	14	14.7	13	13.7	20	21	17	18	16.00^{e}
	Methanol	75	2.4	15	15.8	20	21	20	21	17	18	18.00^{c}

Statistical analysis of variance (ANOVA)

Source	df	*F*-value	*p*-value	R²
Corrected Model	79	206.7	0.0001	0.99
Intercept	1	207734		
Isolates	3	559.2		
Solvent	4	891.9		
Treatment	3	2308.5		

IZ= Inhibition zone diameter (mm), **Conc.**= concentraction, **df**= degree of freedom, ***F***= corresponding level of significance, ***p***= corresponding level of significance, **R²**= Determination coefficient. [a,b] Values having small letters in the same column with different superscripts demonstrate a significant difference, and values with the same letter do not significantly differ, according to Duncan's at $p \le 0.05$.

3.5. Analysis of ethanolic Sage extract using GC-MS

The phytochemical components of the ethanolic Sage extract from GC/MS analysis were shown in **Figure (2)** and **Table (7)**, introduce their retention time (RT), % peak area (concentration), molecular weight (MW), name of metabolite, and mass spectra. There are 41 phytochemical components (peaks) in the extract. The phytochemical compounds were characterized and

identified in **Table (7)** after the mass spectra of the constituents were compared with the NIST library. The 13-Docosenamide, (Z)- covered area percentage reached its highest level at a retention duration of 41.28 min.

The maximum area percentage that presented by 13-Docosenamide, (Z)- has been recognized before by [43] for its antiviral activities that extracted from *Putranjiva roxburghii* using ethanol. [44] added that 13-Docosenamide, (Z)- could be reasonable for the antifungal activity of *R. annamalayana.* Whereas gamma-terpinene that recorded the minimum area in the current study has been discussed by [45] for antibacterial properties in both gram-positive and gram-negative bacteria and discovered that this substance is bactericidal for the reason that they destroy the lipid layer of the bacterial outer membrane with a phenolic structure and destroy the bacterial mitochondria causing structural damage and making bacterial cells more permeable. Other compounds such as Phthalic acid and thunbergol, have antioxidant and antibacterial properties [46, 47].

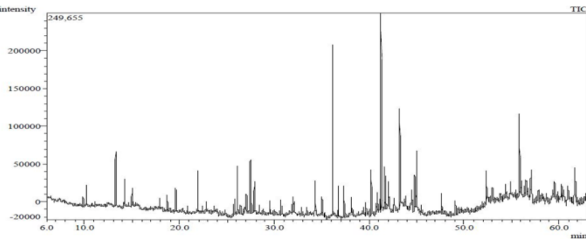
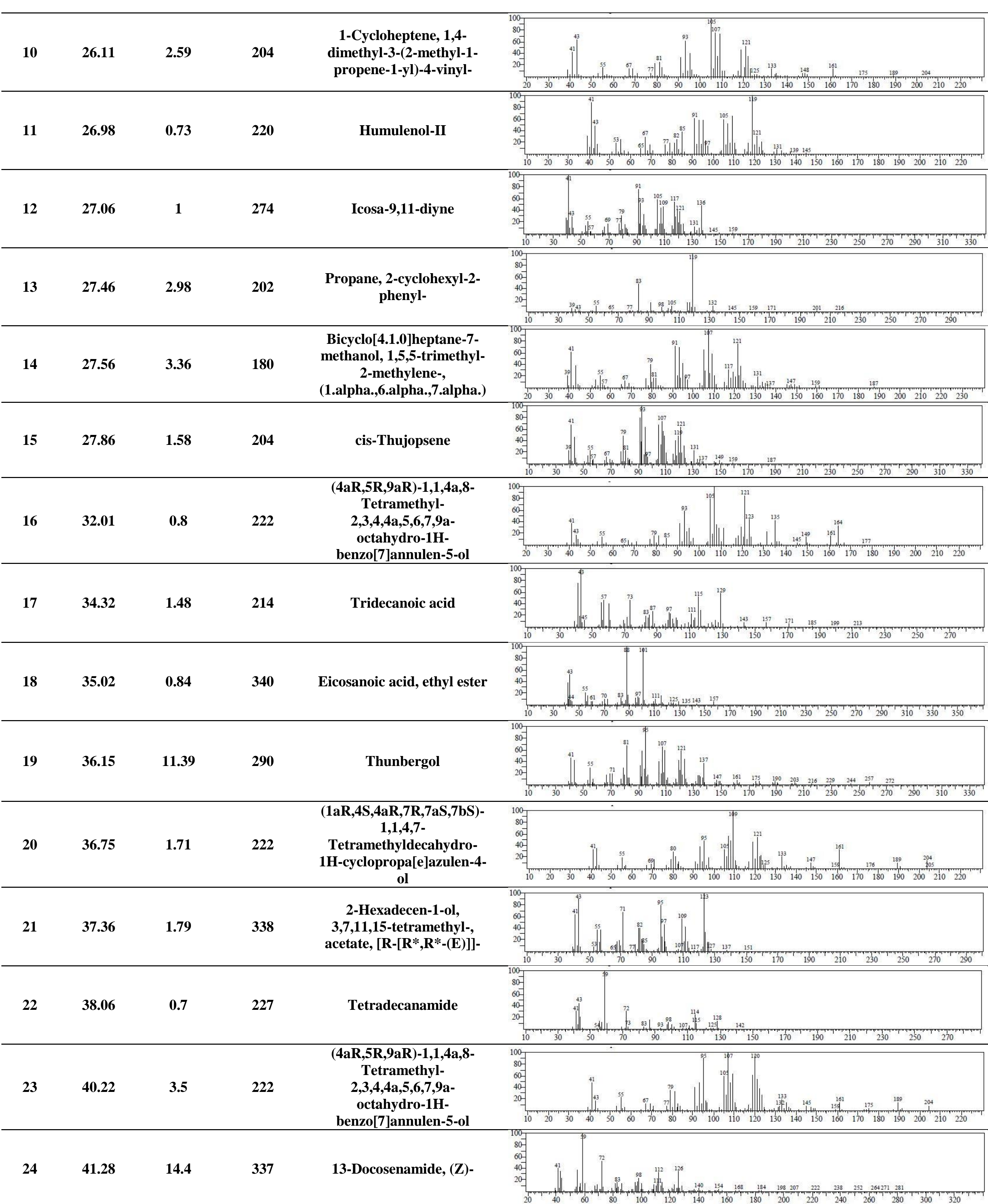


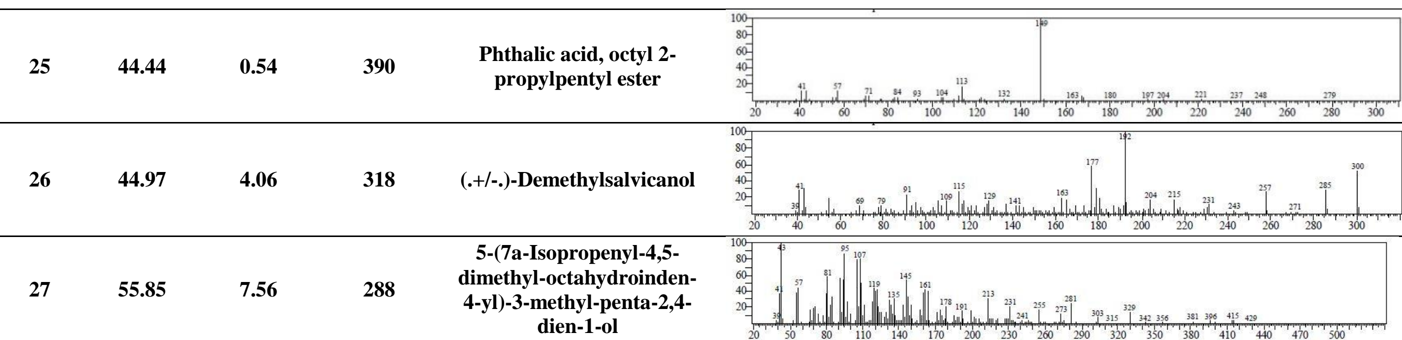
Figure 2: Chromatogram of ethanolic Sage extract by GC-MS.

Table 7
GC/MS analysis of the ethanolic Sage extract.

Peaks	RT (min)	PA (%)	MW (mol)	Name of metabolite	Mass spectrum
1	9.85	0.41	136	.gamma.-Terpinene	
2	10.16	0.99	152	(+)-(E)-Limonene oxide	
3	13.26	2.76	152	Camphor	
4	14.21	1.43	154	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	
5	14.97	0.94	154	.alpha.-Terpineol	
6	18.71	0.9	164	Spiro[2.4]heptane, 1,2,4,5-tetramethyl-6-methylene-	
7	19.58	1.1	164	3-Allyl-6-methoxyphenol	
8	19.71	1.12	196	(1R,3S,4R,5S)-1-Isopropyl-4-methylbicyclo[3.1.0]hexan-3-yl acetate-rel-	
9	21.94	2.11	204	trans-.alpha.-Bergamotene	



No.	RT	Area %	MW	Compound
10	**26.11**	**2.59**	**204**	**1-Cycloheptene, 1,4-dimethyl-3-(2-methyl-1-propene-1-yl)-4-vinyl-**
11	**26.98**	**0.73**	**220**	**Humulenol-II**
12	**27.06**	**1**	**274**	**Icosa-9,11-diyne**
13	**27.46**	**2.98**	**202**	**Propane, 2-cyclohexyl-2-phenyl-**
14	**27.56**	**3.36**	**180**	**Bicyclo[4.1.0]heptane-7-methanol, 1,5,5-trimethyl-2-methylene-, (1.alpha.,6.alpha.,7.alpha.)**
15	**27.86**	**1.58**	**204**	**cis-Thujopsene**
16	**32.01**	**0.8**	**222**	**(4aR,5R,9aR)-1,1,4a,8-Tetramethyl-2,3,4,4a,5,6,7,9a-octahydro-1H-benzo[7]annulen-5-ol**
17	**34.32**	**1.48**	**214**	**Tridecanoic acid**
18	**35.02**	**0.84**	**340**	**Eicosanoic acid, ethyl ester**
19	**36.15**	**11.39**	**290**	**Thunbergol**
20	**36.75**	**1.71**	**222**	**(1aR,4S,4aR,7R,7aS,7bS)-1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol**
21	**37.36**	**1.79**	**338**	**2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]-**
22	**38.06**	**0.7**	**227**	**Tetradecanamide**
23	**40.22**	**3.5**	**222**	**(4aR,5R,9aR)-1,1,4a,8-Tetramethyl-2,3,4,4a,5,6,7,9a-octahydro-1H-benzo[7]annulen-5-ol**
24	**41.28**	**14.4**	**337**	**13-Docosenamide, (Z)-**



25	**44.44**	**0.54**	**390**	**Phthalic acid, octyl 2-propylpentyl ester**
26	**44.97**	**4.06**	**318**	**(.+/-.)-Demethylsalvicanol**
27	**55.85**	**7.56**	**288**	**5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-dien-1-ol**

RT= Retention time, PA= Peak area, MW= Molecular weight.

3.6. Cytotoxicity of ethanolic Sage extract on Vero cell line

Vero is a typical CCL-81 kidney cell line. Those cells adhere to adhering epithelial cells [48] . The cytotoxicity activity of an ethanolic Sage extract towards the Vero cell line was assessed using the MTT technique at six different doses in this work. The Vero cell maintained a percentage of viable cells that varied from 99.90 to 99.95% after getting exposed to an ethanolic Sage extract at a concentration of up to 50µg/ml for 24 hours, according to the data presented in **Figure (3a).** Because of this, the ethanolic Sage extract failed to cause any cytotoxicity in the cell line at doses of 6.25, 12.6, 25, and 50 µg/ml. Cell viability was decreased to 83.3% (causing 16.69% inhibition) at a concentration of 100 µg/ml of ethanolic Sage extract. However, cytotoxicity was not detected because more than 50% of the cells were still alive. When the extract of an ethanolic Sage was applied at a high concentration of 200µg/ml, the cell viability was significantly decreased to 46.34%, and the toxicity increased to 53.65%. The IC_{50} values were calculated as 50% cell viability inhibition [49]. Vero cells had an IC_{50} value of 190.67 ± 4.23µg /ml and a high R^2 of 0.95 **(Figure 3a).**

Using an inverted phase-contrast microscope (OMAX, USA) with a magnification of 100x, the morphological changing of the cell line were studied after treatment with different doses of ethanolic Sage extract (range from 6.25 to 200µg/ml) **(Figure 3b).** The Vero cells survived and had typical adherent cells after being exposed to an ethanolic Sage extract of up to 50µg/ml. While the appearance of the cells changed noticeably, especially at higher concentrations (200µg/ml) of the ethanolic Sage extract, it appears that "apoptotic cells (cell shrinkage)", cell debris, in addition to a significant decrease in the number of cells; indicate cell death in comparison to the control. In this concentration, more than 50% of the cells were still alive, so it has no toxic effect on cells.

The cytotoxic impact of the ethanolic extract of *Glycyrrhiza Glabra* on the Vero cell line has been investigated by [50]. The viability rate of untreated cells was 100%. At 570nm, a UV-spectrophotometer was utilized to identify the live cells. The percentage of viability of the cells was found to be 80% and 83%, respectively, at the highest concentrations 1000µg/ml and 500µg/ml of *G. Glabra*, respectively, exhibiting no cytotoxic activity. *Justicia spicigera* extract did not show significant toxicity in Vero cells (IC_{50} = 54.91µg/ml) [51]. In accordance with [52], the IC_{50} for wormwood ethanolic leaf extract towards the normal kidney cell line was 500µg/ml. The ethanolic leaf extract of *Eucalyptus camaldulensis* has been shown to have cytotoxic effect in vitro towards the normal human fibroblast cell line OUMS, with an IC_{50} value of 165.9µg/ml±10.3 [53].

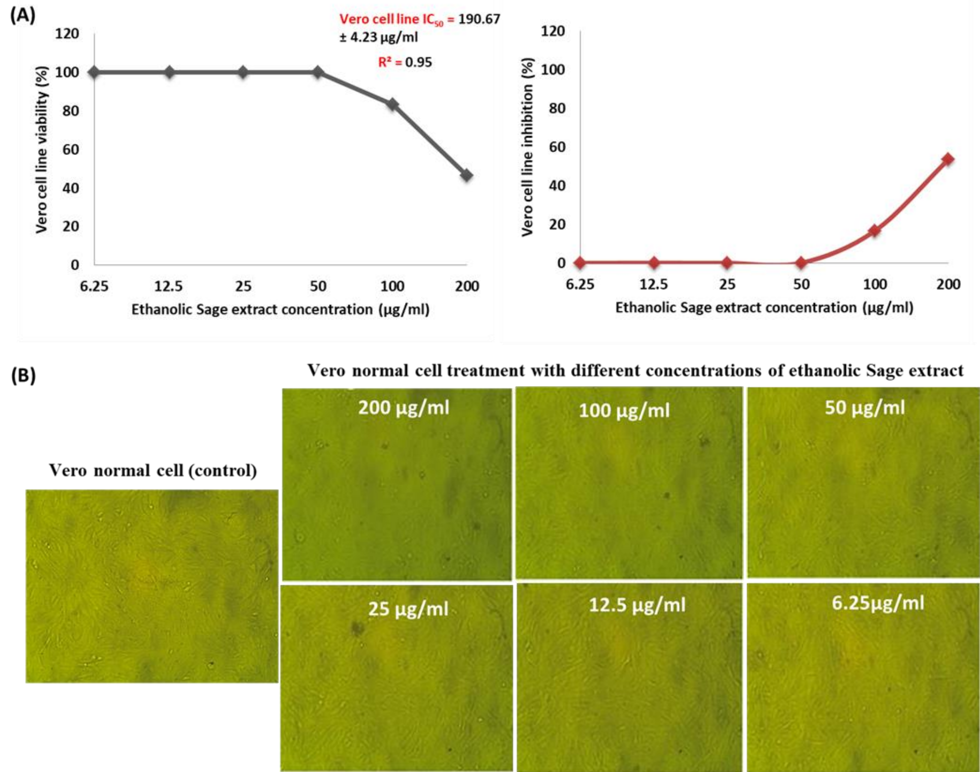


Figure 3: Vero normal cell line viability and inhibition percentage (a) and morphological changes of the cell line (b) after treatment with various concentrations of ethanolic Sage extract (ranged from 6.25 to 200 µg/ml), photographed with an inverted phase-contrast microscope at a magnification of 100×.

4. Conclusion

It could be concluded that the plant aquatic extracts inhibit all G^{+ve} tested bacteria except Black seeds and Cinnamon extracts, affecting only the growth of *S. aureus* strains. Also the *Enterobacteriaceae* G^{-ve} tested bacteria show higher resistance to the effect of aquatic extracts. On the other hand, the inhibition zone of ethanolic extracts was improved to reach 19.8mm by Sage, followed by Cinnamon and marigold against G^{+ve} also G^{-ve} bacteria have the same trend. Organic solvent affects plant extracts' antibacterial activity and yield, and all ethanolic extracts recorded the utmost clear zone and yield percentage. The lowest percentage of yield extract of all tested plants was obtained by chloroform followed by acetone. Ethanolic Sage extract has distinct phytochemical components, with 13-Docosenamide, (Z)- having the highest area with percentage of 14.4%, according to analysis utilizing gas chromatography and mass spectrometry. The normal Vero cell line was not toxified by the ethanolic Sage extract at doses up to 100µg/ml, while the IC_{50} value was 190.67±4.23 µg/ml.

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