



Phytochemical characterization and antibacterial evaluation of crude saps from medicinal plants in Palestinian cuisine

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Abstract

The emergence and evolution of antibiotic-resistant bacterial strains has shifted interest in herbal therapy. Plants have long been a source of anti-infective compounds. This study investigated the antibacterial activity and phytochemical composition of various Palestinian plants against five human pathogenic bacteria. Antibacterial activity was determined using the Agar Diffusion Method (ADM), as well as the Minimal Inhibitory Concentration (MIC) and Broth Microdilution (BMD) assays. The successful ADM bacterial inhibition zones employing plant extracts were *Eucalyptus camaldulensis* Dehn. (*E. camaldulensis*) 9.35 mm, *Allium sativum* L. (*A. sativum*) 8.35 mm, *Ceratonia siliqua* L. (*C. siliqua*) 7.85 mm, and *Amygdalus communis* L. (*A. communis*) 7.85 mm. The MIC₅₀ values against the tested microorganisms varied from 5.69 to 398.5 mg/mL. Furthermore, the MIC₅₀ values for Gram-positive bacteria varied from 5.69 to 233.88 mg/mL, whereas the values for Gram-negative bacteria ranged from 28.65 to 398.5 mg/mL. Most species of bacteria were effectively inhibited by *E. camaldulensis* and *A. sativum* extracts. For Gram-positive and Gram-negative bacteria, the MIC₅₀ values for *E. camaldulensis* were 39.01-40.38 and 31.60-85.37 mg/mL, respectively. The MIC₅₀ values for *A. sativum* against Gram-positive and Gram-negative bacteria were 5.69-14.85 mg/mL and 38.98-200.11 mg/mL, respectively. The phytochemicals such as flavonoids, steroids, proteins, carbohydrates, and alkaloids in varying amounts, may explain their diverse capability to inhibit bacteria. The present study showed that *E. camaldulensis*, *A. sativum*, *C. siliqua* and *A. communis* are valuable Palestinian medicinal plants that contain antibacterial agents against the tested bacterial species. However, this study serves as a foundation for further pharmaceutical studies.

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1. Introduction

Infectious diseases remain the main concern in the world as human-threatening pathogens are present at all times and places. An important contributing factor to this problem is the global spread of antibiotic-resistant bacteria, which poses a major risk to public health worldwide not just through infectious disease outbreaks but also through antibiotic resistance epidemics (1–3). The problem of the emergence and development of antibiotic-resistant strains of bacteria has caused a shift in interest in herbal medicine. Focus has also been placed on studying and analyzing what these plants contain of effective substances against bacteria and using them as alternatives in the treatment of diseases (4,5).

Plants have long been a source of anti-infective compounds. Emetine, quinine, and berberine remain highly effective antibacterial agents. Herbal remedies have demonstrated

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promise in the treatment of severe infectious conditions such as HIV opportunistic infections. Traditional medicine such as protopyrpyrines and related alkaloids, picralima-type indole alkaloids, and Garcinia biflavonones have been demonstrated as effective against a wide range of bacteria (6). Worldwide, there has been a renewed interest in natural products due to the consumer's belief that natural products are superior as well as dissatisfaction with traditional medicines (6,7).

Based on data from the World Health Organization (WHO), medicinal plants are considered valuable source of medicine. Approximately, 80% of people from developed countries use traditional medicine, which contains compounds derived from medicinal plants. However, such plants should be investigated to better understand their characteristics, safety and health efficiency (8). Folk medicine is spreading among the many kinds of people in Palestine. *A. sativum*, *C. siliqua*, *E. camaldulensis* and *A. communis* are among the plants commonly used in herbal stores, and many people use them to treat diseases.

The tested bacteria listed in Table 1 (9–11), were selected in the current investigation because they are likely to be extensively distributed in Palestine due to poor health standards and a lack of public health awareness (12). In addition, those human pathogenic bacteria are the interest and the target of many researchers in Palestine (13). This research is complementary and a continuation of the study note presented at the conference (13,14), in which a more in-depth study was conducted using methods for measuring the antibacterial efficacy of plants and using statistical analysis to compare them. Qualitative and quantitative analysis of the phytochemicals were also performed. Since Folk medicine is not based on scientific foundations, the current research is conducted to shed light on the antibacterial activity of sap extracted from those plants against five species of notably pathogenic bacteria of the human race (Table 1).

Table 1. Diseases caused by the studied bacterial species.

Bacteria	Disease	Reference
<i>Proteus vulgaris</i> (<i>P. vulgaris</i>)	Urinary tract infection	(9)
<i>Klebsiella pneumoniae</i> (<i>K. pneumoniae</i>)	Pneumonia in human	(10)
<i>Escherichia coli</i> (<i>E. coli</i>)	Urinary tract infection, and some strains cause traveler's diarrhea and serious foodborne illness	(10)
<i>Staphylococcus aureus</i> (<i>S. aureus</i>)	Infection of surgical wounds and toxic shock syndrome as well as food poisoning	(10)
<i>Bacillus subtilis</i> (<i>B. subtilis</i>)	Infection of central nerve system and pyogenic meningitis	(11)

2. Materials and Methods

2.1. Sample Collection

Fresh leaf samples of *A. communis*, *C. siliqua* and *E. camaldulensis* and samples of *A. sativum* cloves were collected from different Palestinian regions including Jenin (32.48333°N 35.3°E), Nablus (32.26667°N 35.26667°E) and Tulkarm (32°18'40"N 35°01'51"E) cities. The plants were identified according to Ali-Shtayeh and Jamous (15).

2.2. Sap Preparation

The laboratory work was done in both Microbiology and Biochemistry laboratories/ Department of Biology and Biotechnology/ The Arab American University of Palestine. Samples from each plant were dried for 3 hours at 45 °C and soaked for 2 hours in sterile distilled water (1:20 (w/v)) (16). The suspension was pressed and filtered through fine-mesh sieves then clarified by low-speed centrifugation at 2000 g for 4 minutes. The supernatants (about 100 mL each) were collected and transferred into clean falcon tubes labeled, then stored frozen at –20 °C. The supernatants were dried at 45 °C, weighed and resuspended in sterile normal saline (0.9% NaCl) to obtain a concentration of 1000 mg/mL.

2.3. Preparation of Bacterial Inoculum

The antimicrobial activity of plant extracts was evaluated using five bacterial strains of which were American Type Culture Collection (ATCC); *P. vulgaris* (ATCC 8427), *K. pneumonia* (ATCC 13883), *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) *B. subtilis* (ATCC 21332). The tested bacteria were cultured separately in sterile Mueller-Hinton broth then incubated at 37 °C. Inoculum was obtained by taking colonies from 24 hours cultures. Colonies were suspended in sterile saline (0.9% NaCl) and shaken vigorously for 15 seconds. Density was adjusted to turbidity according to a 0.5 McFarland standard (equivalent to 1–5 x 10⁸ colony forming units per milliliter (CFU/mL) for bacteria (17) with some modifications.

2.4. Seeding of Petri Plates

Seeding was done separately in different Petri dishes containing Mueller-Hinton agar by transferring 0.5 mL of the bacterial suspension into the surface of each plate, then the total volume was spread using a sterile hockey stick glass rod (18).

2.5. Antibacterial Activities of Plant Saps Against a Bacterial Mixture

Bacterial mixture of the previously mentioned bacterial types was prepared at an even ratio by which each bacterium forms one-fifth of the total volume. The mixture was stirred vigorously and stored for 30 minutes at 37 °C. Seeding of the bacterial mixture was done as pointed above. The antibacterial activity of the plant saps was evaluated according to the ADM (10). Hence, 30 µL of plant extract diluted to 100 mg/mL was added to each sterile filter-paper disc (12.7 mm diameter) before being placed on seeded Petri plates. The plates were then incubated at 37 °C for 24 hours, and the zones of bacterial inhibition (between the disc center and the edge of bacterial growth) were determined. Control samples were made by dipping the discs in sterile normal saline in the same way.

2.6. Antibacterial Activities of Plant Extracts Against Bacterial Species

The antibacterial activities of the plant sap were studied against the tested bacterial species listed above (Table 1). Separate cultures of bacterial species grown in Petri dishes from Muller-Hinton agar as described above. Plant extracts were tested against bacteria, either alone or in combination mixed in equal proportions according to the principles of probability, which states that the saps of each other participate in all possible combinations. Antibacterial activity was evaluated in Petri dishes as pointed above.

2.7. Minimal Inhibitory Concentration (MIC) and Broth Microdilution (BMD) assays

Antibacterial activity assays were performed using flat bottom 96-well microtiter plates (19). Bacterial cell suspensions were prepared in Muller-Hinton broth with an optical density equivalent to 0.5 McFarland standard. Negative control samples of broth only and positive control samples of broth inoculated with bacterial cells were also included in each plate. Serial dilutions of each sample of plant extracts were prepared to obtain final concentrations ranging from 100 to 12.5 mg/mL. Therefore, 20–2.5 μ L of plant extract per well was added along with 20 μ L of bacterial culture and volumes of Mueller-Hinton broth to give a total volume of 200 μ L. After 24 hours of incubation at 37 °C, bacterial growth was observed by visual inspection in the wells and compared with positive and negative controls. Absorbance was measured with a microliter plate reader at 570 nm. MIC was determined using the following formula (20,21): Percentage of inhibition = $1 - (\text{Optical density (OD) test} / \text{OD Positive Control}) \times 100$. In addition, the MIC50 was calculated based on the linear equations of the standard MIC curves versus the concentration of the plant extracts. MIC was performed only for the saps of the tested plants separately without combination as the results of the ADM showed neither synergistic nor accumulative effect of the mixtures.

2.8. Sample Preparation for Phytochemical Analysis

Fresh leaf samples of *C. siliqua*, *A. communis*, *E. camaldulensis*, and *A. sativum* were oven-dried at 52 °C for 24 hours. After crushing, the dry samples were soaked with distilled water to make 1:5 (w/v) concentration, then centrifuged at 2000 rpm for 5 minutes. The supernatants were collected and stored at 4 °C for further work.

2.9. Qualitative Phytochemical Analysis

The presence of biologically active substances in plant extracts have been tested using the following standard methods:

2.9.1. Test for Phenols and Tannins

2 mL of 2% solution of FeCl_3 was mixed with crude extract. The presence of phenols and tannins was indicated by a blue-green or black coloration (22).

2.9.2 Alkaline Reagent Test for Flavonoids

2 mL of 2% solution of NaOH was mixed with the crude extract. Flavonoids were indicated by formation of an intense yellow color that becomes colorless with the addition of a few drops of dilute acid.

2.9.3 Test for Saponins

5 mL of distilled water in a test tube was mixed with crude extract and shaken vigorously. The stable foam (frothing) formation was considered as an indication for the presence of saponins (23).

2.9.4. Test for Steroid

2 mL of chloroform was mixed with the crude extract and the concentrated H_2SO_4 was added sidewise. The resulting red color in the chloroform substrate indicates the presence of steroids (22).

2.9.5. Test for Alkaloids

2 mL of 1% HCl was thoroughly mixed with the crude extract and gently heated. Then Mayer and Wagner reagents were added to the mixture. The presence of alkaloids was indicated by the turbidity of the resulting precipitate (23).

2.10. Quantitative Phytochemical Analysis

The amount of biologically active substances in the plant extracts was tested using the following standard methods:

2.10.1. Reducing Sugar Content

1 mL of each sample was added to 1 mL of Benedict's reagent and dipped in boiling water for 5 minutes. Color change was observed and the absorbance at 537 nm was measured for each tube using a spectrophotometer. The concentration of reducing sugar was estimated using a standard curve generated using GraphPad prism 8. 2-5% of pure aqueous glucose solutions were used as positive control (24).

2.10.2. Nonreducing Sugars

190 μ L HCl was added to 1 mL of each sample and heated in boiling water for 5 minutes, the pH of each sample was neutralized with 190 μ L NaCl and the Benedict test was done as pointed above. The concentration of nonreducing sugar was calculated using a standard curve generated using GraphPad prism 8.

2.10.3. Total Protein

Ninhydrin reagent was added to each sample (1:1 (v/v)) and mixed well. The samples were dipped in boiling water for 3 minutes and the violet coloration was observed and measured in spectrophotometer at 357 nm. The concentration of protein was calculated using a standard curve generated using GraphPad prism 8. Lysine (1-5%) solutions were used as positive control reference (25).

2.10.4. Total Starch

Lugol's test was used for the samples to determine the amount of starch (26), pure starch solutions (10^{-5} - 10^{-2}) were used as positive control references and treated alike. Sample coloration was measured at 357 nm using a spectrophotometer. The concentration of starch was calculated using a standard curve generated using GraphPad prism 8.

2.10.5. Total Phenolic Content

Folin-Ciocalteu reagent method was used to determine the total amount of phenol in the aqueous extract (21,27). 1 mL of plant extract was mixed with 2 mL of 2% solution of Na_2CO_3 and 2.5 mL of 10% Folin-Ciocalteu reagent at room temperature. 15 minutes later, the absorbance was measured at 765 nm using gallic acid as a standard. The tests were performed twice. The standard curve used to determine the results that were expressed as gallic acid equivalent (1 mg GAE/L of extracted compound).

2.11. Statistical Analysis

Each experiment was conducted in triplicates, results and values are expressed as mean \pm SD. Multiple comparisons were performed by one-way ANOVA followed by Dunnett's test

for antimicrobial activity of plant saps and two-way ANOVA for polyphenol content using GraphPad PRISM 8. Significance was established at $P < 0.05$.

3. Results and Discussion

3.1. Antibacterial Activities of Plant Saps Against a Bacterial Mixture

Evaluation of the antibacterial activities based on the filter disc method showed that plant saps extracted from *E. camaldulensis*, *A. sativum*, *C. siliqua* and *A. communis* had antibacterial activities against the bacterial mixture. Moreover, both *E. camaldulensis* and *A. sativum* showed the strongest antibacterial activity among the four tested plants. These plants showed inhibition zone diameters of 9.35 and 8.35 mm, respectively (Table 2, Figure 1).

Table 2. Zones of ADM produced by the tested plant extracts.

Common Name	Scientific Name	Parts Used	Zone diameter (mm)
Garlic	<i>Allium sativum</i> L.	Cloves	8.35
Almond	<i>Amygdalus communis</i> L.	Leaves	7.58
Carob	<i>Ceratonia siliqua</i> L.	Leaves	7.85
Eucalyptus	<i>Eucalyptus camaldulensis</i> Dehn.	Leaves	9.35

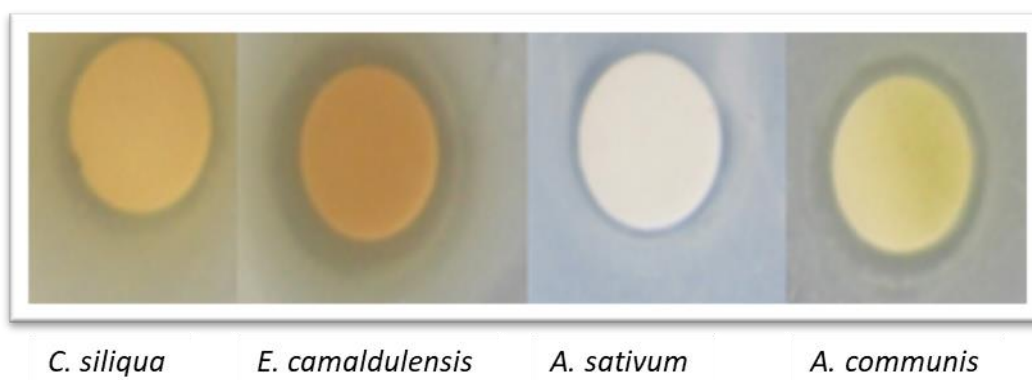


Figure 1. ADM showing the zones of bacterial growth inhibition.

3.2. Antibacterial activities of plant saps against bacterial species

The results revealed that the extracts of *A. sativum* and *E. camaldulensis* have the maximum antibacterial activity against most tested bacterial types. In addition, the mixture of *E. camaldulensis* and *A. sativum* have a strong ability to inhibit most species of the tested bacteria. Furthermore, the mixture of *C. siliqua* and *A. sativum*, the mixture of *C. siliqua*, *E. camaldulensis* and *A. communis* and the mixture of *A. communis*, *A. sativum*, *C. siliqua* and *E. camaldulensis* have significant results as antibacterial agents against most tested bacterial species. The other treatments revealed an intermediate efficiency against bacteria except the *A. communis* and the mixture of *A. communis* and *E. camaldulensis* that have the lowest effect (Figure 2).

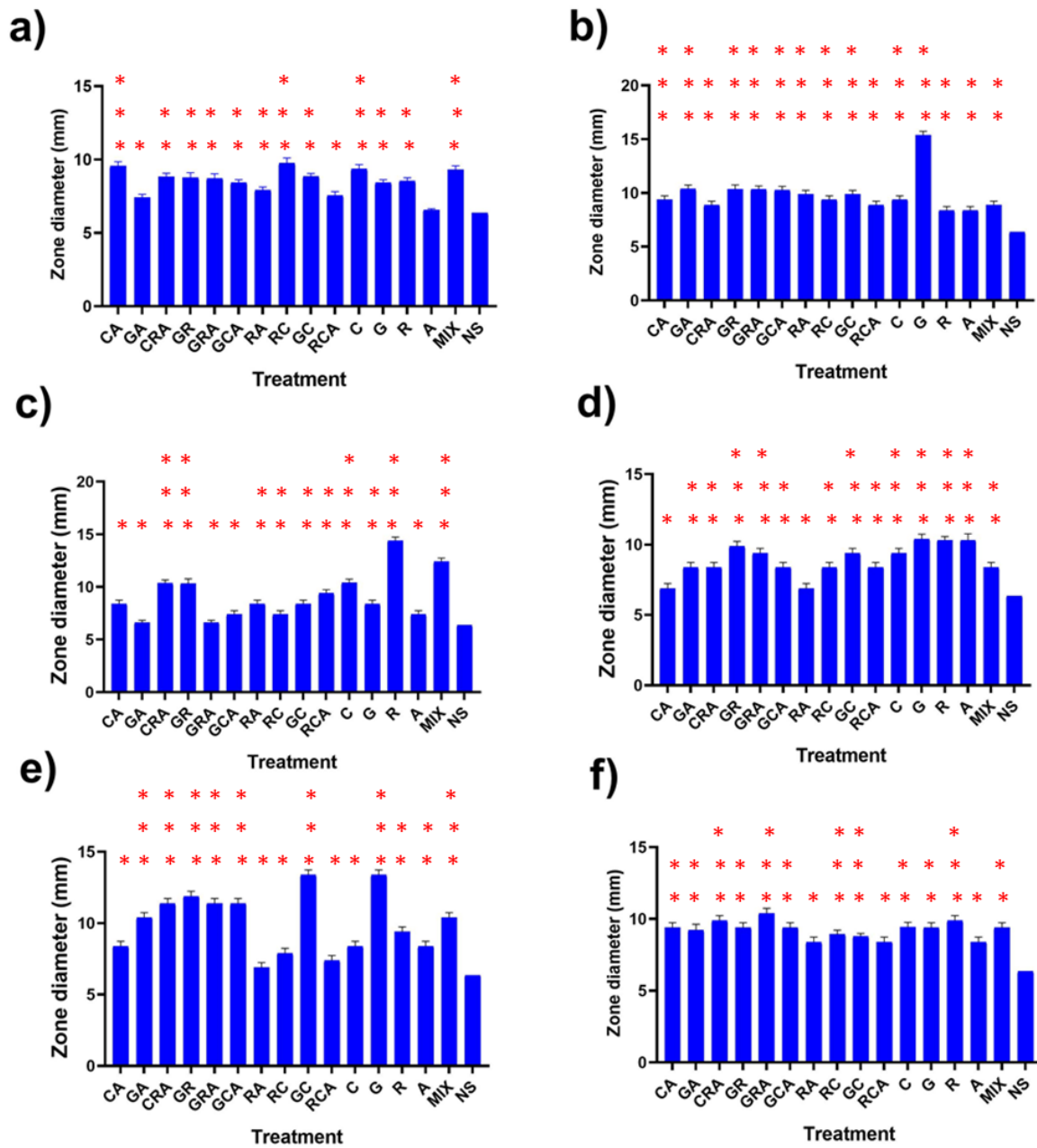


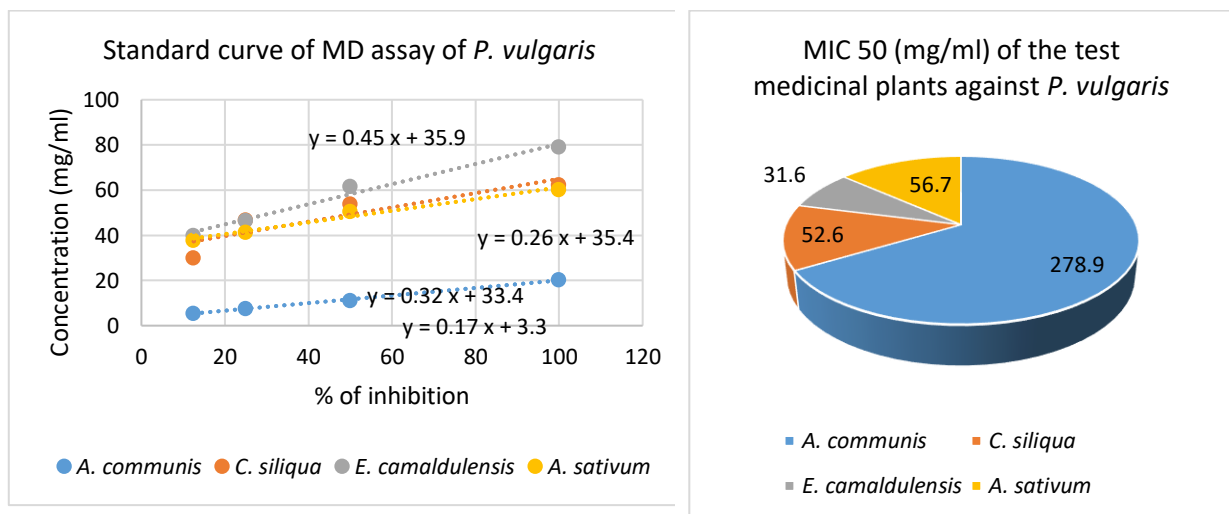
Figure 2. Antimicrobial activity of plant extracts against a) *K. pneumoniae*, b) *B. subtilis*, c) *P. vulgaris*, d) *E. coli*, e) *S. aureus* and f) bacterial mixture. For extracts (G) represents *A. sativum*, (C) *C. siliqua*, (A) *A. communis* and (R) *E. camaldulensis*. (MIX) represents all extracts mixture, (NS) for normal saline. Each bar represents mean \pm SD of three different experiments carried out in triplicates. The asterisk indicates statistically significant difference from control which was calculated using a one-way ANOVA followed by Dunnett's multiple comparisons test. (* $p < 0.05$ vs. LPS, ** $p < 0.01$ vs. LPS, and *** $p < 0.001$ vs. LPS).

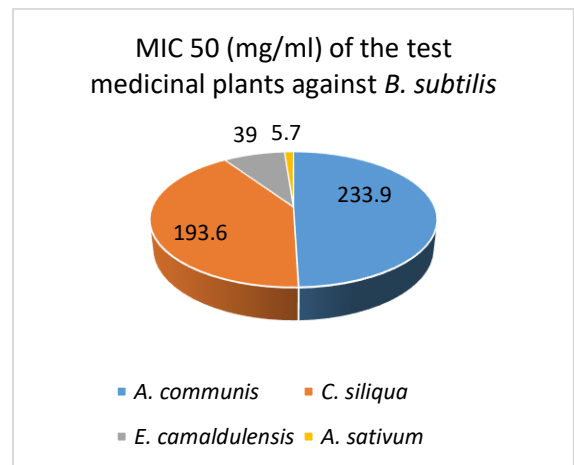
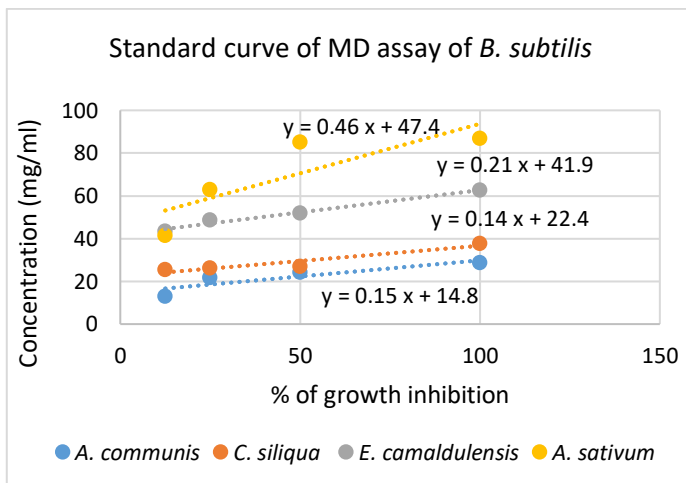
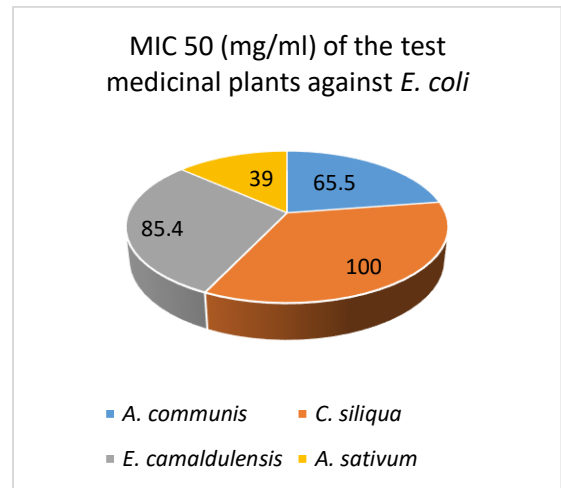
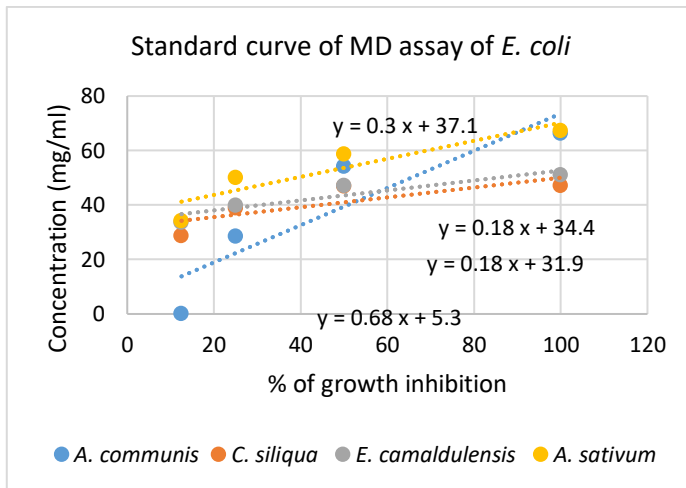
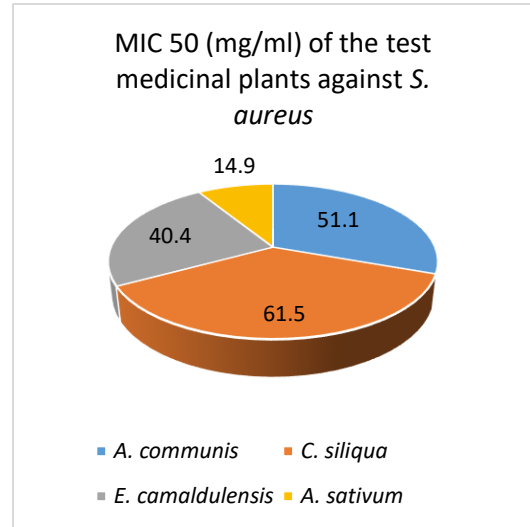
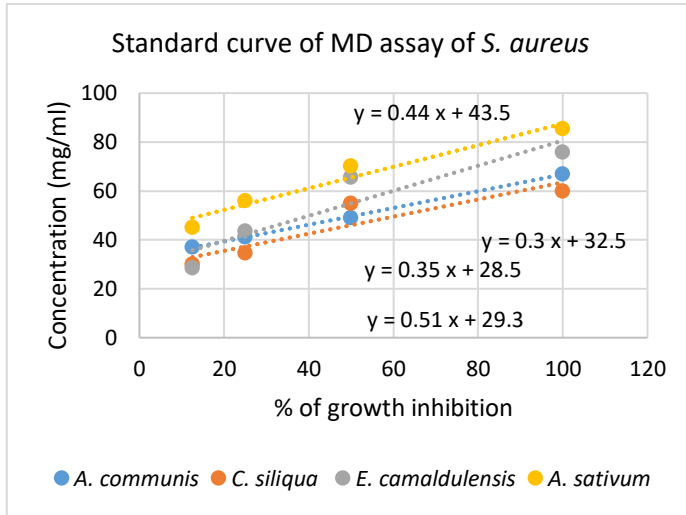
In addition, the results showed that *A. sativum* and the mixture of *A. sativum* and *C. siliqua* have the maximum antibacterial effectiveness against *S. aureus*, whereas, the mixture of *A. communis* and *E. camaldulensis* revealed the minimum ability against the bacterium. *E. coli* was efficiently inhibited by *A. communis*, *A. sativum*, and *E. camaldulensis* and also by the mixture of *A. sativum* and *E. camaldulensis*. *P. vulgaris* was strongly inhibited by the *E.*

camaldulensis, whereas, the other saps revealed either an intermediate or low inhibition value against it. *B. subtilis* was strongly inhibited by *A. sativum* and the other saps showed an intermediate or low inhibition for this pathogen. For the *K. pneumoniae*, most saps revealed an intermediate inhibition except the *A. communis*, which showed the lowest inhibition value (Figure 2).

3.3. Broth Microdilution Assay

The results of BMD assay showed that all examined plants are with good efficacy against the tested bacteria, where when calculating the MIC50 based on the standard curves, these values ranged from 5.69-398.5 mg/mL for all tested plants against bacterial species that were studied (Figure 3). In addition, the values of MIC50 against Gram-positive bacteria were ranging from 5.69-233.88 mg/mL, while the values of the same assay for Gram-negative bacteria were ranging from 28.65-398.5 mg/mL. It was found from the results obtained from MIC that *E. camaldulensis* and *A. sativum* extracts were generally effective against most types of bacteria. The MIC50 of *E. camaldulensis* ranged from 39.01-40.38 and from 31.60-85.37 mg/mL for Gram-positive and Gram-negative bacteria respectively. For *A. sativum*, the MIC50 against Gram-positive and Gram-negative were 5.69-14.85 and 38.98-200.11 mg/mL respectively. *B. subtilis* was highly sensitive to *A. sativum* and *E. camaldulensis* extracts, with MIC50 values of 5.69 and 39.01 mg/mL, respectively. Also, the extracts of these two plants were superior in effect against *S. aureus*, as the MIC50 values were 14.85 and 40.38 mg/mL, respectively (Figure 3).





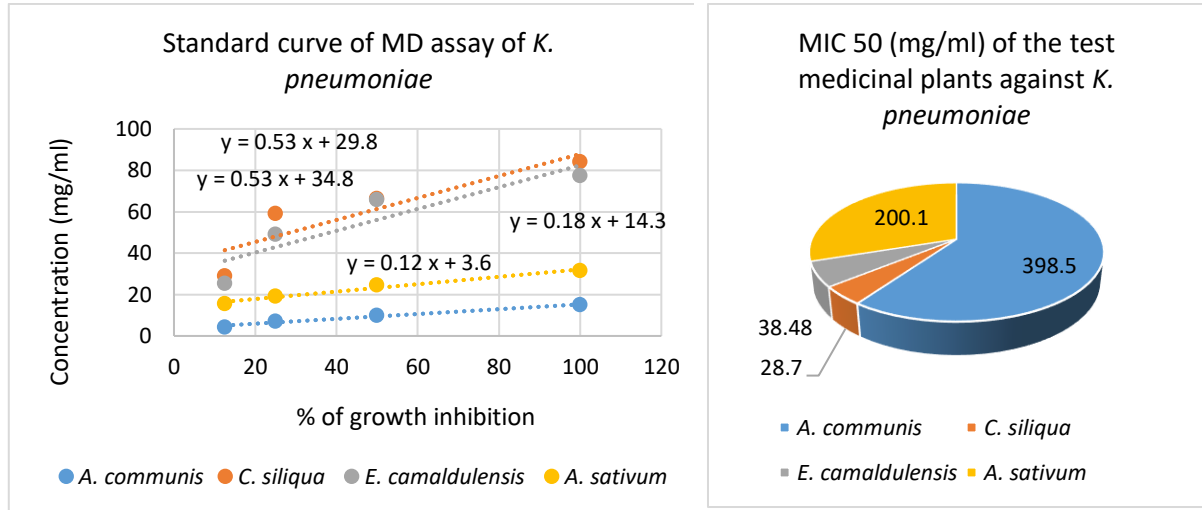


Figure 3. Standard curves of BMD and MIC50 of plant extracts against the tested bacteria.

For Gram-negative bacteria, *K. pneumoniae* was sensitive to extracts of *C. siliqua* and *E. camaldulensis* leaves, as the MIC50 values for them were 28.65 and 38.48 mg/mL, respectively. *A. sativum* and *A. communis* leaf extracts were effective against *E. coli*, as their MIC50 values were 38.98 and 65.49 mg/mL, respectively. As for *P. vulgaris*, *E. camaldulensis*, *C. siliqua*, and *A. sativum* were the most effective against it, as the MIC50 values for them were 31.60, 52.62, and 56.71 mg/mL, respectively (Figure 3).

Plant antimicrobials represent a large, untapped resource for medicines that have enormous therapeutic potential. Plant antimicrobials are effective in treating infectious diseases while at the same time alleviating many of the side effects often associated with synthetic antimicrobials (28). Plant-derived medicines are showing great contributions to mankind in treating infections of pathogenic bacteria (29). The results illustrated that the human pathogenic bacteria may be inhibited by natural products from the plants growing in Palestinian environment. In this regard, the results revealed that *A. communis*, *C. siliqua*, *E. camaldulensis* and *A. sativum* are effective medicinal plants to inhibit the growth of the tested bacteria. These plants must contain quantities of antibacterial phytochemicals that enable them to have this effect. Also, the combination mixtures from these plants were also effective against the tested bacteria, this means that their active materials are not antagonistic and can work together to achieve the antibacterial effects. In this regard, Gupta and Ravishankar (30) showed that *A. sativum* paste is a potent antibacterial against *E. coli* O157:H7. The use of garlic extract reduced the likelihood of quality degradation in tuna fillets during storage (31). *A. sativum* has an excellent antimicrobial effect against intestinal bacteria, including *E. coli*, and is recommended for patients with gastroenteritis (32).

It has been demonstrated that *A. sativum* possesses antibacterial properties that protect against a variety of bacteria, including strains that are resistant to drugs (33). *A. sativum* antibacterial properties come from its organosulfur components, particularly allicin (34). Additionally, *A. sativum* extracts can increase the effectiveness of various medicines against drug-resistant bacteria, including ciprofloxacin and gentamycin (33). *A. sativum* was the most often used herbal remedy among diabetes patients, according to a study done in Palestine, which also improved their lipid profiles and blood glucose levels (35). According to reports, certain Gram-positive and Gram-negative bacteria, including *P. aeruginosa*, *B.*

subtilis, *S. aureus*, and *E. coli*, are susceptible to the antibacterial activity of *A. communis*. The phenolic chemicals found in *A. communis*, including epicatechin, gallic acid, and catechin, are responsible for their antibacterial properties. In Palestine, *A. communis* oil has also long been used as a traditional treatment for wounds and skin ailments (36). Antibacterial activity of *C. siliqua* against pathogenic microorganisms like *Vibrio cholerae*, *Shigella sonnei*, and *Salmonella typhimurium* has been observed. Robusta's antibacterial properties are associated with its tannins, flavonoids, and saponins. Palestinians grow and eat a lot of *C. siliqua*, which has been used to heal sore throats, coughs, and diarrhea (37). It has been shown that *E. camaldulensis* possesses antibacterial activity against certain bacteria that cause respiratory infections, including *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. The essential oil of *E. camaldulensis*, which includes limonene, alpha-pinene, and eucalyptol, is what gives it its antibacterial properties. Palestine also grows *E. camaldulensis*, which has been used to treat fever, bronchitis, and asthma with its leaves and blooms (38).

3.4. Qualitative Phytochemical Analysis

Table 3 represents the phytochemical characteristics of the tested plants with antibacterial activity. The medically active compounds were detected in most tested plant samples including phenols, flavonoids, steroids, proteins, carbohydrates, and alkaloids. Saponins were absent in all the plants. Phenols were detected in high amount in both *E. camaldulensis* and *C. siliqua*. Steroids were absent only in the leaves of *A. sativum* and *E. camaldulensis*. Carbohydrates were detected in all tested samples while proteins were absent only in *E. camaldulensis*.

Table 3. Phytochemical constituents of the tested medicinal plants.

Plant	<i>A. communis</i>	<i>A. sativum</i>	<i>E. camaldulensis</i>	<i>C. siliqua</i>
Phenols	+	+	+++	+++
Flavonoids	+	+	+	+
Saponins	-	-	-	-
Steroids	+	-	-	+
Proteins	+	+	+	+
Carbohydrates	+	+	+	+
Alkaloids	-	+	-	-

A positive sign (+) represents a low amount while (+++) a high amount of the compound. (-) represents its absence

3.5. Quantitative Phytochemical Analysis

The quantitative Benedict's reaction showed that *A. communis* extract contained the highest amount of sugar, amounting to 72.71 mg/g, followed by *E. camaldulensis* containing 67.77 mg/g, then *C. siliqua* and *A. sativum* with amounts of 60.91 mg/g, 48.12 mg/g, respectively. For non-reducing sugar, the highest content was in *A. sativum* 81.25 mg/g, then *C. siliqua* 77.61 mg/g. In addition, Lugol's reaction showed that *E. camaldulensis* leaf extract is the most starch-containing extract 25.22 mg/g, followed by *A. communis*, *A. sativum* and *C. siliqua* in decreasing order. For the protein content in these plants, Ninhydrin test showed similar amounts of protein in these plants, the highest of which was in *A. sativum* (44.04

mg/g), then *E. camaldulensis*, *A. communis*, and *C. siliqua*, respectively (Table 4). The total phenolic contents obtained by the Folin-Ciocalteu reagent method were 338, 39.2, 86.5, 338 mg GAE/L for *E. camaldulensis*, *A. sativum*, *A. communis* and *C. siliqua* respectively. *E. camaldulensis* and *C. siliqua* revealed much higher content of the total phenol compared with the other tested plants, whilst *A. sativum* showed the lowest phenol content of the tested plants (Figure 4).

Phytochemical analysis revealed the presence of components known to have medicinal effect as well as antibacterial activities (39). Regarding the total phenol content, the results showed that *E. camaldulensis* and *C. siliqua* contain high amounts of phenols, and this probably explains their great ability to inhibit bacteria. In addition, the activity of *A. sativum* and *A. communis* on bacteria can be explained by the fact that they contain certain amounts of the same substances. Phenolic compounds are one of the largest and most abundant compounds of plant metabolites (40). Beside their biological properties such as antitoxic, antiaging, anticancer, many phenolic compounds may exhibit significant antibacterial activity. Furthermore, it was proved that polyphenolic compounds have antibacterial properties that may exhibit significant antibacterial activity against both Gram-positive and negative bacteria as well as foodborne pathogenic or food-spoiling bacterial strains (41). Flavonoids are known to be antibacterial agents against a wide range of pathogenic microorganisms (42). Their varying antibacterial properties can be explained by the fact that all of the plants under study contain flavonoids. One possible explanation for the activity of flavonoids is their capacity to form complexes with extracellular and soluble proteins that are complexed with bacterial cells (43). On the other hand, the studies have shown the role of steroids as antibacterial agents, and this explains the ability of plants such as *A. communis* and *C. siliqua* to inhibit bacteria, as the results revealed that these plants contain certain amounts of such chemicals (44).

The results showed that plants contain abundant amounts of protein and carbohydrate, which supports their different abilities in inhibiting bacteria. As a type of the defense response, plants manufacture toxic molecules, including antimicrobial peptides, to kill or inactivate pathogens by interacting with phospholipids and permeabilizing the membrane. Some peptides have cell penetrating action which are able to introduce many cargoes into cells in the absence of specific receptors by interacting with membrane phospholipids (45–48). Such peptides found in roots, seeds, flowers, stems, and leaves have been reported to have activities against bacteria that are also pathogenic to humans (49). In addition, certain carbohydrates may exhibit antibacterial activity when extracted from plants. Fructose extracted from *Vaccinium* spp exhibited antibacterial activity (28). Glycosides together with other phytochemicals extracted from *Oroxylum indicum* leaves exhibited antibacterial activities against *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* (50,51).

Table 4. Total amount of phytochemical in plant extracts.

Plant	Non-reducing	Starch	Benedict	Ninhydrin
<i>E. camaldulensis</i>	75.34±2.1	25.11±4.5	67.77±4.6	43.92±3.2
<i>A. sativum</i>	81.25±3.3	15.06±2.2	48.12±2.6	44.04±4.2
<i>A. communis</i>	59.51±1.7	20.50±3.2	72.71±2.2	41.43±4.3
<i>C. siliqua</i>	77.61±2.2	14.69±4.2	60.91±1.9	40.76±2.6

*conc mg/g (Interpolated)

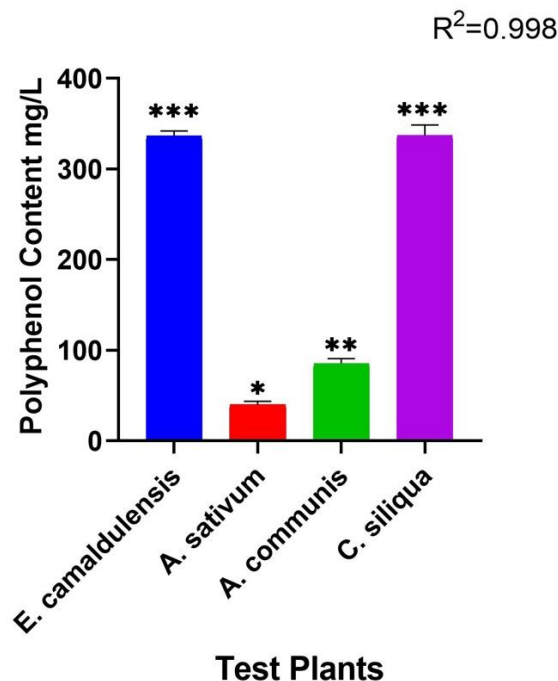


Figure 4. Polyphenol content mg/g of *E. camaldulensis*, *A. sativum*, *A. communis* and *C. siliqua* plants. Each bar represents mean \pm SD of three different experiments carried out in triplicates. Asterisk indicates statistically difference in means which was calculated using two-way ANOVA.

The results showed that the Gram-positive bacteria including *B. subtilis* and *S. aureus* were affected by the plant extracts more than the Gram-negative ones including *P. vulgaris*, *K. pneumoniae* and *E. coli*. Generally, most Gram-negative bacteria are not susceptible to substances as the Gram-positive bacteria. The lower susceptibility of Gram-negative bacteria may be attributed to the composition of the cell wall which consists of one or a very few layers of peptidoglycan and the outer membrane which consist of lipopolysaccharide, lipoprotein and phospholipids. Also, the outer membrane acts as a barrier that may inhibit the entry of antimicrobials and the Gram-negative bacteria have fewer peptide cross-bridges (10,52). Therefore, *P. vulgaris*, *E. coli* and *K. pneumoniae* showed relative resistance against antibacterial agents in plant saps as they possess Gram-negative cells with outer layers. On the other hand, Gram-positive bacteria as *B. subtilis* and *S. aureus* showed a significant inhibition by different plant saps. This finding may be attributed to the structure of the Gram-

positive cells that are liable and easily destroyed by antimicrobials and other chemicals which occur naturally in some plant cells (10).

4. Conclusions

The present findings demonstrated the importance of native flora as sources of antibacterial agents against bacteria for human life. The results also revealed the possibility of using these plants against bacteria that infect humans and using them in the pharmaceutical industry for new drug preparations. Since these substances come from natural resources, the risk of their side effects on human health and the environment may be minimal. Therefore, their use may fully or partially replace the use of synthetic antibacterial drugs with harmful side effects. The results of the ADM were consistent with the results of the MIC in determining the antibacterial effect of the plant sap. The ADM was suitable for measuring and comparing the antimicrobial activity of plant sap, while the MIC is more suitable for determining the accurate concentration of the antibacterial substance which can be used for further clinical and pharmacological studies. Gram-positive bacteria were more sensitive to the test plant extracts than Gram-negative bacteria, based on MIC₅₀ values. Additionally, *E. camaldulensis* and *A. sativum* extracts were typically efficient against most bacteria, according to MIC values. However, this study will likely provide scientific data that will enable the application of these plants as herbal remedy with antibacterial properties and serve as a foundation for further pharmaceutical research.

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Author Contributions

H.S. Conceptualization, Writing- Original draft preparation, Reviewing and Editing, Supervision, Resources. S.K. Reviewing, Resources. B.M. Reviewing, Formal analysis. Y.A. Investigation, Reviewing. Z.A.A. Investigation, Reviewing. A.K. Validation Reviewing and Editing. All authors contributed to the study concept and design. All authors gave final approval to the submitted paper, and agreed to be accountable for all aspects of the work.

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Institutional Review Board Statement

Not applicable.

Data Availability Statement

The data that support the findings of this study are openly available in Zenodo at <http://doi.org/10.5281/zenodo.7812260>.

Conflicts of Interest

The authors declare that they have no competing interests.

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