



Antibacterial Efficacy of Extraction of *Salvia palaestina* Bentham

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ABSTRACT

Salvia palaestina is native to Iraq and other Mediterranean Regions, but is now grown worldwide, primarily for use as a culinary herb. It is a member of the Lamiaceae family. Plant specimens were collected in different areas of MRO (Rowanduz mountain district), MSU (Suleimany mountain district), FPF (Persian foothill district) and FKI (Kirkuk foothill district) of Kurdistan Region-Iraq from mid- March 2022 the end of June 2022. Flavonoids and phenols were found in the study's preliminary screening for the key phytochemical natural product groups. Furthermore, a study of the antibacterial anti-bacterial Efficacy of *Salvia palaestina* Bentham leaves of three concentrations of 25%, 50%, and 100% mg/ml by hydro-alcoholic (ethanol) extract against four types of bacteria pathogenic to humans was used in the study preserved and diagnosed. They were obtained from the Zanko Medical Laboratory. Two Gram-Positive pathogenic bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), and two Gram-Negative (*Escherichia coli* and *Pseudomonas aeruginosa*) were studied. The results revealed that the impact of hydro-ethanol extract in concentration of 100% mg/ml in *Salvia palaestina* a 41.66 mm inhibition zone was the most effective against bacteria *Pseudomonas aeruginosa* compared to all studied extract concentrations. The average extract a 30.6 mm inhibition zone was more potent than the average control (Ciprofloxacin) a 23.5mm in inhibition the growth of the studied bacteria. Also, the studied Antibiotic (Ciprofloxacin) did not affect *Staphylococcus aureus*. *Staphylococcus aureus* isolated resistant to Ciprofloxacin. This study revealed that the anti-bacterial efficacy of *Salvia palaestina* leaves by hydro-ethanolic extract had more vigorous anti-bacterial activity than control treatment 2 (Ciprofloxacin).

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Keywords: Phytochemistry, Lamiaceae, Anti-Bacterial Activity, *Salvia Palaestina*, Flavonoids, Inhibition Zone.

1. Introduction

Salvia of the Lamiaceae family was the most prominent genus, with about 900 species in the world^[1]. These plants were usually 30-150 cm tall, herbaceous, perennial, seldom biennial or annual, with attractive flowers in a variety of hues, and get their name from the Latin word "salvage" "about the therapeutic and curative abilities of *Salvia* plants^[2]. *Salvia palaestina* grows in various habitats, at elevations ranging from 1,000 to 4,000 feet (300 to 1,220 m). This plant grows to 1 to 2 feet (0.30 to 0.61 m) tall, erect, and has numerous square-shaped stems that grow from basal roots. Its green leaves, which were corrugated and wrinkled, vary in shape and size. These leaves had light hairs on both sides, containing glands that release a scent and aroma when rubbed or crushed. At the apex of the stems, the inflorescences reach a length of 12 inches (30 cm), are candelabra-shaped, and

have four to six flowers per panicle. The flowers are upright, tubular, and grow to be approximately 0.5 inches (1.3 cm) long. They are also white to pale lilac in color^[10]. Accordingly, different *Salvia* species were the subject of extensive phytochemical and pharmacognostic research for the isolation, and characterization of their secondary metabolites^[3]. Some secondary metabolite conducts execute physiological functions^[4]. The major phytochemical constituents in *Salvia* species include diterpenoids, phenolic acids, triterpenoids, flavonoids, and saccharides. Flavonoids, triterpenoids, and monoterpenes were mostly distributed in the aerial parts of the plants, particularly in the flowers and leaves, whereas phenolic acids and diterpenoids were primarily found in the roots^[5]. Moreover, several groups had isolated and identified the structures of flavonoids possessing anti-bacterial activity. Additionally, several studies demonstrated a beneficial interaction between active flavonoids and already available chemotherapeutics^[6]. Phenolic acids were natural plant metabolites known for their numerous biological activities^[7]. Better-known compounds from this group were Rosmarinic Acid

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(RA), an ester of Caffeic, and 3, 4-Dihydroxyphenyllactic Acids. It was additionally found to have an anti-bacterial impact against plenty of naturally occurring species of Gram-Positive and Gram-Negative bacteria as well as an interaction with some drugs against antibiotic-resistant strains^[8]. Phenolic acids (e.g., Salvin and Salvin monomethyl ether) isolated from *salvia* had anti-microbial activities, especially against *Staphylococcus aureus*^[9]. The primary objective of this study was to evaluate the hydro-ethanol plant extract of *Salvia palaestina* Benthham in order to anti-bacterial activity and phytochemical screening. The primary objective of this study was to evaluate the hydro-ethanol plant extract of *Salvia palaestina* Benthham in order to anti-bacterial activity and phytochemical screening.

2. Materials and Methods

Plant collection:

Plant specimens (Figure 2) were collected in different areas of {MRO (Rowanduz mountain district), MSU (Suleimany mountain district), and FPF (Persian foothill district and FKI (Kirkuk foothill district)) of Kurdistan Region-Iraq. From mid-March 2022 to the end of June 2022. In this study, *S. palaestina* (leaves) were collected and placed it in nylon bags with the necessary herbarium information recorded (place and date of collection, name of the collector, and growth stage with some ecological and field notes) were recorded. Then transferred to the laboratory some of which were dried and pressed for diagnosis. At the same time, others were cleaned of dust and impurities. Then rinsed with water and dried at room temperature away from light (to avoid photo-oxidation). After drying, the plant is refrigerated until use. In this study, *S. palaestina* can be identified according to some morphological features based on the Flora of Turkey^[10]. And Flora of Iranica^[11]. Is a perennial herb with opposing pairs of ovate-lanceolate leaves with jagged edges and velvety or hairy leaves. Its hollow square stem is 30–60 cm tall, branches from the base, and has long, soft hairs. Flowers that are up to 2 cm long. The plant's distinctive flower had a violet-blue calyx around a white corolla. Corolla upper lip falcate^[10] (Figure 2).

Extraction method:

The hydro-alcoholic (ethanol) extract was prepared by mixing 20gm of plant powder in 200ml of ethanol (70:30 alcohol: water), with stirring (by 1:10 weight: volume), after that leave the mixture in the refrigerator for 24 hours to soak. The extract was filtered through several layers of gauze and then filtered again using Whatman filter paper No. 1 to remove the non-pulverized plant parts and the remaining fibers. Then, it was placed in a rotary evaporator at a temperature 40 C. After evaporation of the alcohol in the mixture, a thick layer of the extract was obtained, and then it was placed in a shaker incubator at a temperature of 25-30 C. After, drying the obtained extracts were stored at 4 C in airtight containers protected from light and moisture with information written on them until further analysis^[12].

Phytochemical screening:

Phytochemical screening in this study was based on using two reagents: sodium hydroxide reagent and ferric chloride FeCl_3 reagent (Table 1).

Table 1: chemical detection of active compounds in *Salvia palaestina*.

Active compounds	Detection method	Detection result	<i>S. palaestina</i>
	Used reagent		
Flavonoids	Sodium hydroxide	which becomes colorless on addition of HCL	+
Phenols	Ferric chloride	Green color	+

Detection of Flavonoids:

Detection was prepared by adding two drops of sodium hydroxide reagent into one ml of the plant extract. The formation of an intense yellow color, which becomes colorless with addition of dilute acid (HCL), indicates the presence of flavonoids^[13].

Detection of Phenols:

Detection was prepared by adding 3 ml of plant extract to 2 ml of ferric chloride reagent FeCl_3 . The appearance of a bluish-green color indicates the presence of phenolic compounds^[14].

Inoculum Preparation and Preparation of Studied Bacterial Suspensions:

Since the disk diffusion approach used phenotypic susceptibility identification, the following steps were necessary:

1. Prepared an inoculum from a standardized bacterial culture:
 - Selected colonies in remote locations.
 - Prepared a bacterial suspension (inoculum).
 - By utilizing McFarland standards, the bacterial solution was standardized.
2. Giving one of the following a bacterial suspension inoculation:
 - A specific growing medium is Mueller Hinton Agar (MHA).
 - Disks with antibacterial properties were added (just for disk diffusion).
 - Plate incubation.
 - Measured the inhibition zone.

The diffusion agar method was used by well to observe the sensitivity of two Gram-Positive pathogenic bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), two Gram-Negative (*Escherichia coli* and *Pseudomonas aeruginosa*) of the extract of the studied plant at concentrations (25%, 50%, and 100% mg/ml). The streak plate method, involves incubating the inoculated Muller-Hinton broth-MHA (containing fast-growing bacteria) for 2 to 6 hours. In general, the "direct colony suspension method" was used to prepare inoculum from colonies produced within 18 to 24hours^[15]. The examined bacteria were individually cultured on sterile Muller-Hinton Agar (MHA) for 24 hours at 37°C. The growth was transferred into a sterilized test tube containing 5 ml of sterile standard saline solution^[15]. The bacterial suspension in the test tubes was thoroughly and uniformly stirred using a vortex mixer. Then, 0.5 McFarland turbidity standards were used to modify the bacterial suspension.

By comparing them with a 0.5 McFarland turbidity equivalence standard with a white backdrop and contrasting blue lines, the inoculum tubes' turbidity was adjusted and compared^[16]. Before inoculating bacterial suspension in a growth medium, make sure there was not too much inoculum on Mueller Hinton Agar (MHA). This was done by spreading 100 microliter (μL) of the bacterial suspension on the MHA plates. The agar wells were created using a 6 mm diameter sterilized cork borer, with 3 wells per plate. Three different concentrations (25% mg/ml, 50% mg/ml, and 100% mg/ml) of the plant extract solutions were carefully applied to the corresponding wells in the plate media by a micropipette and a tip (for one-time use) 50 μL /well. Before incubation, the plant extracts and antibiotic disc were allowed to diffuse and then left at room temperature for 30 minutes in order to take place the impregnation. The plates were then incubated at a temperature of 37 °C for 18-24 hours for bacteria^[16]. Finally, measurements were taken every two days, and the diameter of the inhibition zones was measured in millimeters (mm) using Caliber after an overnight incubation^[17].

Two controls were prepared as a control treatment (the first positive for bacteria and the second negative for bacteria), as following:

1. The control treatment was prepared previously; Antibiotic was adopted as a positive control factor when Ciprofloxacin (CIP-10 mg) was used for bacterial positive control. By using a dispensing tool (sterile pair of forceps). The antibiotic disc was applied to the surface of the inoculated agar plate and pressed down to ensure total contact with the agar surface. Three replicated for each treatment (studied bacterial species), two Gram-Positive pathogenic bacteria (*E. faecalis* and *S. aureus*), and two Gram-Negative (*E. coli* and *P. aeruginosa*).
2. In the previous method, with except for placing instead of the plant extract in the well. By using a micropipette (50 μL /well) of sterilized distilled water (control was negative) as used in the study. Three replicated for each treatment studied bacterial species: two Gram-Positive pathogenic bacteria (*E. faecalis* and *S. aureus*), and two Gram-Negative (*E. coli* and *P. aeruginosa*)^[18].

3. Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) R software, and the Tukey test was used to test out the differences among treatments (controls, bacteria, and concentrations)^[19].

4. Results

Chemical detection of active compounds:

The phytochemical screening in (leaves) of *S. palaestina* extract by hydro-ethanol indicated the presence of flavonoids and phenols (Table 1).

Anti-bacterial efficacy of *Salvia palaestina* extract:

The results showed that hydro-ethanol extract had a negative effect on the growth of (*E. coli*, *E. faecalis*, *P. aeruginosa*, and *S. aureus*) at concentrations of 25%, 50%, and 100% mg/ml, as determined by the diameter of the inhibition zone in (mm) in comparison to the controls 1 and 2 (Table 2). Comparing all examined extract concentrations, a concentration of 100% mg/ml in *S. palaestina* produced a 41.66 mm inhibitory zone, which was the most potent against the bacteria *P. aeruginosa* (Figure 1). There were no appreciable variations in concentrations of 25% and 50% mg/ml with 22.33 and 24.33mm inhibition zones, respectively (statistical p value = 0.9798987) to against *E. coli*. In comparison to controls 1 and 2, the concentration of 100% mg/ml with a 40.66mm inhibition zone was the most effective to against *E. faecalis*. The inhibition zones for the concentrations of 25% and 50% mg/ml against *E. faecalis* were 30.33 and 34mm, respectively (Figure 1), showed no appreciable variations between them. The concentrations of 25%, 50% and 100% mg/ml in *S. palaestina* with 22.33, 24.33, and 26.66 mm inhibition zones, respectively, there were no appreciable variations among them to against *E. coli*. In comparison to controls 1 and 2, the average extract of 30.6mm was the most effective in inhibiting the studied bacteria (Table 2). There were no appreciable differences in *S. palaestina* between concentrations of 25% and 50% mg/ml with inhibitory zones of 22.33, and 24. 33mm, respectively. Additionally, there were no significant variations between 50% and 100% mg/ml concentrations with respective inhibitory zones of 24.33 and 27.33 mm against *S. aureus* (Figure 1).

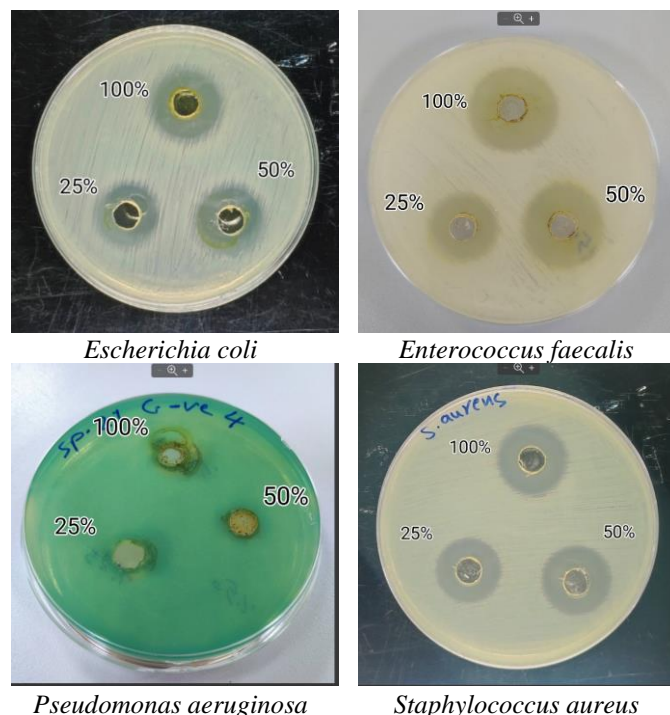


Figure 1: effect of *Salvia palaestina* extract against all studied bacterial species.

Table 2: antibacterial activity of extracts of *Salvia palaestina* (mm). The figures show the average over three replicates. Vertically comparable uppercase letters indicate that there are no statistically significant differences between them at the 0.05 level of probability. Vertical lowercase letters that are similar indicate that there are no appreciable differences between them at the 0.05 level of probability.

Extract	Concentration mg/ml	Bacteria				Average Concentration	Average Extract
		<i>E.coli</i>	<i>E.faecalis</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>		
Hydro- Ethanol	25%	A 22.33 a	B 30.33 d	C 35.33 a	A 22.33 m	27.58 a	30.6 a
	50%	A 24.33 a	BD 34 d	CD 38 a	A 24.33 hm	30.16 e	
	100%	A 26.66 a	BD 40.66 f	CD 41.66 c	A 27.33 h	34.07 c	
	Average bacteria	G 24.44	H 35	M 38.33	N 24.66		
Control	D.W	B 0 b	B 0 b	B 0 b	B 0 b	B 0 b	0 b
	ciprofloxacin	M 40 c	K 14 g	M 40 cd	S 0 b	23.5 d	23.5 c

5. Discussion

In accordance to the results there were differences between the effects of the extracts on the growth of Gram-Positive bacteria and Gram-Negative bacteria. Furthermore, this study confirmed previous observations of the highly different sensitivity of various bacterial species to flavonoids and phenols. Additionally, the studied extract inhibited bactericidal effects against studied bacteria. Flavonoids, which support the plant's anti-bacterial activity, were present in the plant extract used in this investigation. The studied results considered that the extraction method using hydro-ethanol had anti-bacterial activities. Plant family Lamiaceae and the most prominent genus *Salvia* were some of the richest sources of antimicrobial^[20]. Studied results demonstrated that the average extract with a 30.6mm inhibition zone was more robust than average controls with a 23.5mm inhibition zone against studied bacteria (Table 2). May be Due to the fact that the examined phytochemical bio-active compounds of *S. palaestina* comprise flavonoids and phenols (Table 1). Also, it May be due to essential parameters that can affect the ethno pharmacological of the extract, including the age of the plant and the plant part (leaves) used for extraction. Also, the studied antibiotic (ciprofloxacin) did not affect *S.aureus*. *S. aureus* isolated resistant to ciprofloxacin were described due to a gene mutation that occurred in *S.aureus*^[21]. Moreover, *S. aureus* Gram-Positive bacteria was a highly changeable pathogen in response to antibiotics with considerable importance in human medicine^[22]. The ethanol extract was resistant to both Gram-Positive bacteria like *S. aureus* and Gram-Negative bacteria like *K. pneumoniae*^[23]. In the other case (ciprofloxacin) was the most effective against Gram-Negative bacteria than Gram-Positive bacteria (Table 2). Additionally, flavonoids had been discovered to reduce membrane permeability, pathogenicity, porin on cell membranes, adhesion, and biofilm formation, all of which were

essential for bacterial growth^[24]. Flavonoids were present and had a variety of biological effects, including anti-bacterial, cytotoxic, potent antioxidant, and anti-inflammatory effects^[25]. According to the studied result, phenolic compounds present the studied plant extract strongly affected studied bacteria^[26]. Polyphenolics had reportedly been found to possess antibacterial activity in other circumstances^[27]. The studied result agreed with the study considered to be crucial bio-active compound flavonoids present in leaves of *S. palaestina* were showed anti-bacterial solid action against *S. aureus*, *S. epidermidis*, *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*^[28]. Finally, the main factors could be discussed in the studied antimicrobial efficacy that the extracts made from several *S.palaestina* samples displayed significant changes in the flavonoids and phenolic composition, the resulting differences in the investigated biological properties due to the heterogeneity of the environmental conditions. The results strongly indicate that *S.palaestina* leaves are a rich source of essential flavonoids and phenols with exceptional anti-bacterial and characteristics. Particularly those collected from mid-March 2022 to the end of June 2022 (Figure 2). Because of the low rate of environmental pollution, which ensures the best quality of plant material, protected regions, representing the most ecologically clean locations, can be excellent sources of medicinal plants.

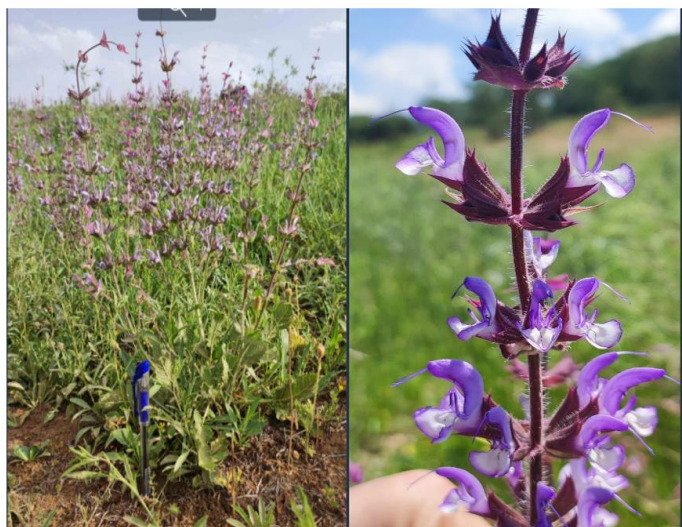


Figure 2: Field pictures of *Salvia palaestina* studied species.

Conclusions

Nowadays, more than 5,000 phytochemicals have been identified, although their composition varies depending on the species of plant. According to recent research, *S. palaestina* leaves contain a wide variety of bio-active phytochemicals with significant therapeutic effects. This study concluded that the anti-bacterial efficacy of *S. palaestina* leaves by hydro-ethanolic extract had more potent anti-bacterial activity than Antibiotics (ciprofloxacin). Ciprofloxacin had low impact on *S. aureus* but showed vigorous effect against *E. faecalis*, *E. coli*, and *P. aeruginosa*. Therefore, this study recommends future research towards separating the bio-active compounds in (leaves) of *S. palaestina*. Using (HPLC) High Performance Liquid Chromatography technology. Detections of further bio-active compounds using different extraction methods (such as methanolic and aqueous).

Conflict of interests

None

Authors contribution

Dina and Sirwan did field trips, collecting and classifying the sample. In addition, analyzing the anti-bacterial efficacy of hydro-ethanol extract of *Salvia palaestina* leaves, the detection of flavonoids and phenols was done by Dina. Mohammed performed the statistical analysis.

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