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Study of the Aqueous and Alcoholic Extracts of Sage Leaves (Salvia officinalis) on the growth of pathogenic bacteria in Sirte City

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Abstract

This study assesses the antimicrobial properties of natural products derived from sage leaves (Salvia officinalis), a member of the Lamiaceae family, against five pathogenic bacterial strains. The investigation employs aqueous and alcoholic sage leaf extracts, prepared using both cold and hot water. The bacterial strains included in the study were Staphylococcus, Streptococcus pyogenes, Escherichia coli, Salmonella, and Shigella. The chemical examination also revealed the presence of significant active components in these extracts, including tannins, saponins, flavonoids, glycosides, resins, and alkaloids. The inhibition results of the extracts exhibited variability contingent on the extraction method, temperature, and solvent employed, thereby reinforcing the assertion that this plant merits consideration as a prospective natural antibiotic. It is therefore recommended that further quantitative and qualitative studies be conducted in order to determine the molecular structure of the active components present and to reduce reliance on synthetic compounds that may have harmful effects on humans.

Keywords: Sage, aqueous and alcoholic plant extracts, some types of pathogenic bacteria.

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Introduction

Medicinal and aromatic herbs are well-known plants that have been used in alternative treatments for a variety of diseases due to the presence of active compounds. As a result of scientific and technological advancement, researchers have been able to accurately identify the active ingredients found in medicinal and aromatic plants, isolate them from the plant, and remanufacture them for use in medical and nutritional fields (Judd, 1997).
The advent of serious illnesses resulting from the overuse of chemical pharmaceuticals has prompted scientists to direct their attention towards the extracts of medicinal plants, given that the majority of these do not induce adverse effects. It has been demonstrated that certain medicinal and aromatic plants contain chemical substances, including essential oils and other active compounds such as alkaloids and glycosides, which have the capacity to treat a range of diseases (Hazendaroglu, 2001). Consequently, there has been a notable increase in demand for drugs extracted from aromatic plants, particularly in recent years.

This has led to the view that any medicinal herb can be considered a complete pharmacy due to the active compounds they contain (Kopilas et al., 2002). The active ingredients have demonstrated efficacy in the treatment of acute and chronic diseases affecting humans, including antidiarrheal and anti-inflammatory properties, the management of cardiovascular conditions, the regulation of blood coagulation, and the treatment of viral infections (Suppakul et al., 2003). Furthermore, they have been employed in the development of treatments against microbes and various types of bacteria, thereby demonstrating their effectiveness. This is due to the fact that some plants are capable of producing aromatic compounds, including saponins and the majority of their secondary derivatives (Kim et al., 1995). As reported by the World Health Organization, approximately 80% of the global population relies on plant-based remedies for treatment (Ribeiro et al., 2018). This statistic substantiates the growing inclination towards alternative medicinal practices that utilise aromatic plants, as opposed to chemically synthesised pharmaceuticals. The objective of this study is to evaluate the inhibitory efficacy of aqueous and alcoholic extracts of sage (Salvia officinalis) against a range of pathogenic bacteria.

Methods

The following materials were employed in the test: a glass rod, flasks, particular-sized glass tubes, dissection sheets, glass beakers, medical cotton, magnetic stirrer, crucible. The following apparatus was utilised in the realistic experiments: a heating mantle, a pH meter, a rotary evaporator, a water tub, a touchy balance. The following chemicals were employed in the realistic experiments: methanol (CH_3OH), sodium hydroxide (NaOH), distilled water, ferric chloride ($FeCl_3$), acetic acid (CH_3COOH), ethyl acetate (CH_3COEt), ammonia solution (NH_3), concentrated hydrochloric acid (Con. The following chemicals were used: hydrochloric acid (HCl), sulfuric acid (H_2SO_4), chloroform (CH_3Cl), and Dragendorff's reagent [$K(BiI_4)$].

The process of collecting and training plant fibres is known as 'coaching'. The plant material is sage (Salvia officinalis), as illustrated in Figure 1.

Figure (1) sage (Salvia officinalis)

The plant was collected and prepared for analysis.

Prior to the flowering stage, samples of the sage plant (Salvia officinalis) were collected in February 2020, which coincided with the practical phase of the study. The samples were gathered from the outskirts of Sirte city.

The cleaning, drying and grinding processes are described in turn below.

The cleaning process entailed the use of water to remove impurities, plant residues, and clay particles. The samples were subsequently dried in a dark place at room temperature, ensuring thorough mixing to guarantee optimal drying and prevent spoilage for a period of 10 to 15 days. Subsequently, the material was pulverised to create a dry powder, which was stored in opaque paper bags for subsequent analysis to detect active chemical compounds and test the biological activity of the plant extracts.

The objective is to identify the active compounds present in the sage plant.

A series of tests were conducted with the objective of identifying the active compounds present in the plant extract. The following tests were conducted:

The detection of flavonoids was achieved through the utilisation of a suitable detection solution, comprising 5ml of ethanol (CH_3CH_2OH) at a concentration of 50% and 5ml of potassium hydroxide (KOH) at a concentration of 50%. Following the mixing of equal quantities, 1 ml of the ethanol and potassium hydroxide solution was combined with 1 ml of the aqueous extract of the sample (Jaffer et al., 1983). The formation of a yellow precipitate in the sage plant filter was observed, indicating the presence of flavonoids (see Figure 2).

Figure (2): Result of the Flavonoid Test for Sage

The detection of tannins was conducted as follows: 5 grams of the dry plant powder was weighed and placed in a 50 mL beaker. Subsequently, 25 mL of distilled water was added, and the solution was heated. Following cooling, filtration was performed, and the filtrate was divided into two portions.

The initial portion of the sample was subjected to the following procedure: A volume of 1 mL of the sample extract was taken and combined with an equal volume of lead acetate. The second part of the procedure is as follows: A volume of 1 mL of the sample extract was combined with an equal volume of 1% ferric chloride (Shihata et al., 1951). The formation of a gelatinous precipitate was observed, as illustrated in Figure 3.

Figure (3) Result of the Tannins Test for Sage

Detection of Resins:

A quantity of 1 g of the dry plant powder is weighed and transferred to a conical flask. Subsequently, 5 ml of ethanol (CH_3CH_2OH) at a concentration of 95% is added. The solution is then boiled for a period of two minutes in a water bath, after which it is filtered. To the filtrate, 10 ml of distilled water acidified with a few drops of hydrochloric acid (HCl) at a concentration of 4% (Fahmy et al., 1933) is added. The formation of turbidity in the sage extract, as illustrated in Figure 4, indicates the presence of resins in the extract.

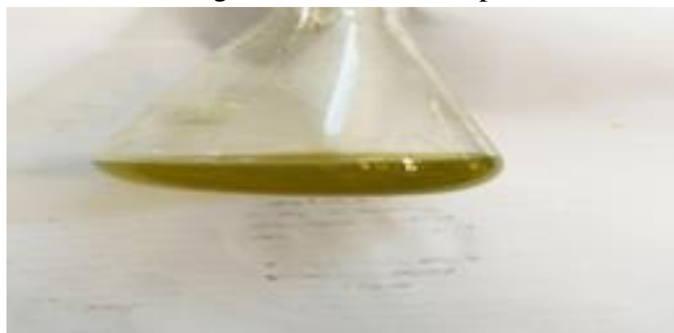

Figure (4): Result of the resin test for sage

Detection of Steroids:

The alcoholic extract of the plant was subjected to evaporation, and the resulting residue was dissolved in 5ml of chloroform (CH_3Cl). Subsequently, the solution was filtered once more in order to remove any remaining impurities, and the resulting filtrate was divided into two portions.

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To the initial portion of the solution, 1ml of acetic acid was subsequently added, followed by 1ml of concentrated sulfuric acid (H_2SO_4), with great care taken to ensure that the latter was added along the wall of the test tube. A further 1 ml of concentrated sulfuric acid (H_2SO_4) was added to the second part of the solution, with the addition being made along the wall of the test tube (Shihata et al., 1951).

The appearance of a yellowish-green colour, which subsequently changes to red (or reddish-brown), was observed in the form of a ring, indicating the presence of steroids in the sage extract (see Figure 5). This suggests the presence of unsaturated steroids in the sage extract.

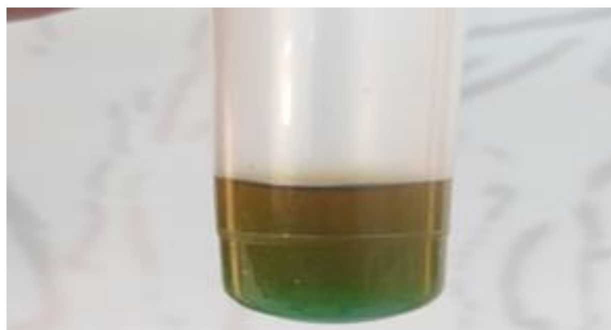

Figure (5) Result of the Steroids Test for Sage

Detection of Alkaloids:

A quantity of 10 g of the dry plant powder is weighed and transferred to a 100 mL beaker, to which 50 mL of distilled water acidified with a few drops of hydrochloric acid (HCl) at a concentration of 4% is added. Subsequently, the solution is heated and then cooled and filtered. The following reagents were used in the testing process:

Mayer's reagent is defined as follows: Two millilitres of the aqueous filtrate of the sample are taken and a few drops of Mayer's reagent (Fahmy et al., 1933) are added. The presence of alkaloids in the plant extract was indicated by the formation of an orange precipitate, as illustrated in Figure 6.

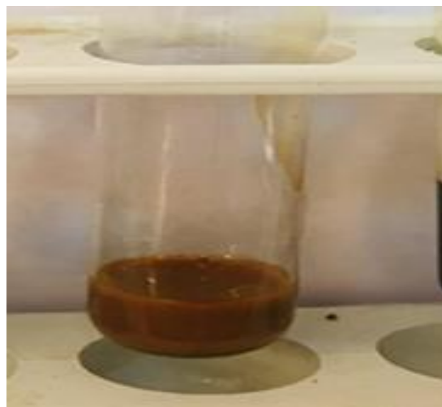

Figure (6) Result of the Alkaloids Test for Sage

Detection of Saponins:

The initial methodology is as follows: A quantity of 1ml is taken from both the aqueous and alcoholic extracts of the plant and transferred to test tubes. These are then shaken for one minute and allowed to stand for 20 seconds.

Second Method for Confirmation: A solution of 1 ml mercuric chloride should be added to 2 ml of both the aqueous and alcoholic extracts of the sample (Shihata et al., 1951). The formation of a dense and stable foam was observed in the plant extract, which is indicative of the presence of saponins (see Figure 7).

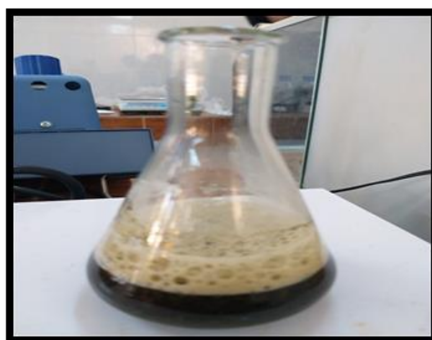

Figure (7): Result of the Saponins Test for Sage

pH Measurement:

The acidity of the plant extracts was determined by conducting a measurement using a pH meter. The device was submerged in flasks containing the aqueous extracts, and the results for each sample were documented. Upon completion of the test, it was observed that the pH of the sage plant was 5, indicating that the aqueous extract exhibited low acidity.

Preparation of Plant Extracts:

In order to prepare the aqueous and alcoholic extracts, a pattern of dried plant powder weighing 10 g was combined with either 50 ml of distilled water for the aqueous extract or methanol for the alcoholic extract. Subsequently, the aggregate was permitted to soak for a period of twenty-four hours at room temperature, after which it was filtered on three occasions. This indicates that filtration was conducted at 24-hour intervals, with distilled water employed for the aqueous extract and methanol for the alcoholic extract (each for a period of three days). Subsequently, the three filtrates were subjected to evaporation (evaporation) using a rotary evaporator, as illustrated in Figure 8. This process yielded a raw extract, which was subsequently stored for future utilisation. Similarly, extracts may be prepared through the application of heat, whereby the extracts are boiled (Jaffer et al., 1988).

Figure (8) rotary evaporator

Biological Study:

A quantitative assessment of the antimicrobial activity of extracts against a range of bacterial strains was conducted. The following pathogenic bacteria were included in the study: The study employed five strains of bacteria obtained from the Faculty of Medicine (Department of Microbiology) at Sirte University. Table 1 provides an overview of these types.

Table 1: Types of Bacteria Used in the Study

NO	Scientific Name	Staining Reaction
1	S. aureus	Positive
2	S. Pyogenes	Positive
3	E. coli	Negative
4	Salmonella	Negative
5	Shigella	Negative

Preparation of Bacterial Suspension (Bacterial Cultivation):

The five test tubes, which have been sterilised using an autoclave, are then placed in a test tube holder. Subsequently, 1ml of distilled water is added to each tube. A plastic loop is employed to extract a sample from the medium in which the bacteria were previously cultivated, ensuring that the loop does not come into contact with the culture medium. The sample is then introduced to the test tubes, which are subsequently agitated with a laboratory shaker until the bacteria are fully incorporated into the distilled water. Subsequently, the tubes are covered with medical cotton and left for a period of 30 minutes, as illustrated in Figure 9.

Figure (9): Preparation of the Bacterial Suspension

The inoculation and incubation of the culture medium employed for sensitivity testing (Mueller-Hinton agar) is described below.
The medium was prepared in accordance with the manufacturer's instructions, whereby 10 g of the medium was dissolved in 250 ml of distilled water. The medium was sterilised via incubation at 100°C for a period of one hour, after which it was permitted to cool for a further half an hour. Ten Petri dishes were prepared and filled with the culture medium to allow it to solidify. The aforementioned medium was employed for the cultivation of bacteria and the placement of extract discs, as detailed below:
The following instructions pertain to the discs. Discs with a diameter of 6 mm were manufactured using a circular paper cutter and chromatography paper with a thickness of 3 mm. The discs were sterilised in an autoclave for one hour at 121°C and then soaked in the extracts until completely saturated. The discs have been numbered and their positions marked at equal distances, with the abbreviated name of the extract written on each.
Bacteria have been transferred to the lifestyle medium using a swab, and the swab has been unfolded across the entire surface of the dry lifestyle medium in overlapping strains. This process has been repeated three times, with the Petri dish rotated at a 60° angle between each repetition to ensure that the microorganism grew throughout the complete medium. This process was repeated for all bacterial traces.
The saturated chromatography paper discs were then placed into the Petri dishes with the aid of sterile forceps and incubated for a period of twenty-four hours at a temperature of 37°C, as evidenced in the referenced source (10). This procedure was conducted in accordance with the method described by Túnez et al. (2011), which involved the use of agar medium.

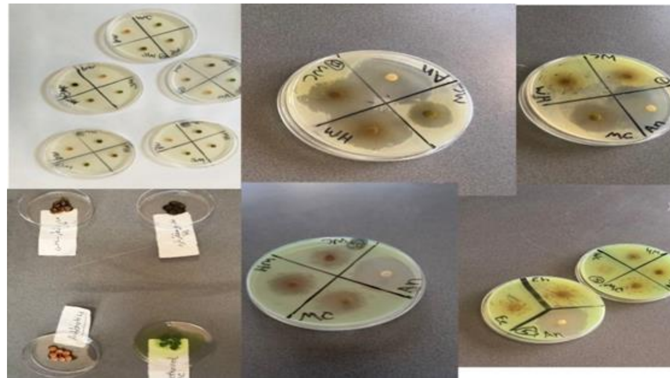

Figure (10): Steps for Planting and Incubating the Growth Medium

Results of the Biological Activity Study:

The following is a summary of the results of the biological activity test of sage extracts against five types of bacteria. The results of the antagonism test demonstrated a degree of variability between different extracts, contingent on the solvent utilized. The impact of the plant extracts on the bacteria was evaluated in comparison to the antibiotic, which served as the control, using cold water as the solvent. The extract exhibited the greatest efficacy against Salmonella, with an inhibition zone diameter of approximately 3.5 cm, followed by Shigella, which had an inhibition zone diameter of 2.2 cm. The effects on other bacterial types were as follows: The inhibition diameter for E. coli was observed to be (1.5 cm), while that for S. pyogenes was (1.1 cm). Nevertheless, no effect was observed on S. aureus, as illustrated in Figure 11.

Table (2): Results of Inhibition Zone Diameters of Extracts Against Bacteria

NO	Bacteria	Extracts			
		W.c	W.h	Me.c	Antibiotic
1	S. aureus	0	0.7cm	3cm	4cm
2	S. pyogenes	1.1cm	0.7cm	1cm	3.3cm
3	E. coli	1.5cm	0.7cm	0.65cm	3cm
4	Salmonella	3.5cm	3.4cm	2.2cm	4cm
5	Shigella	2.2cm	2.2cm	1.8cm	3.5cm

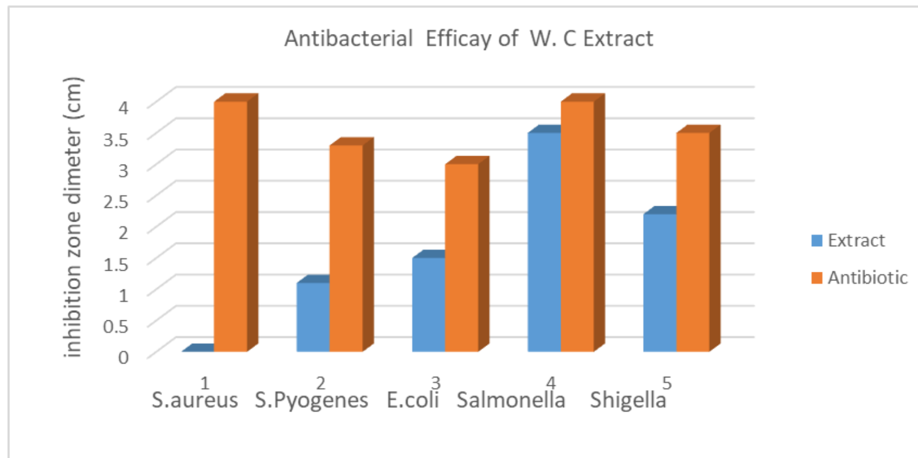

Figure (11): Inhibition Zones of Cold-Water Extract of Sage Against Bacteria

The aqueous extract of the plant exhibited the most pronounced inhibitory effect against Salmonella, with an inhibition zone diameter of 3.4 cm, followed by Shigella with an inhibition zone diameter of 2.2 cm. Meanwhile, the inhibition zones for S. aureus, E. coli, and S. pyogenes were found to be approximately equal at 0.7 cm, as illustrated in Figure 12.

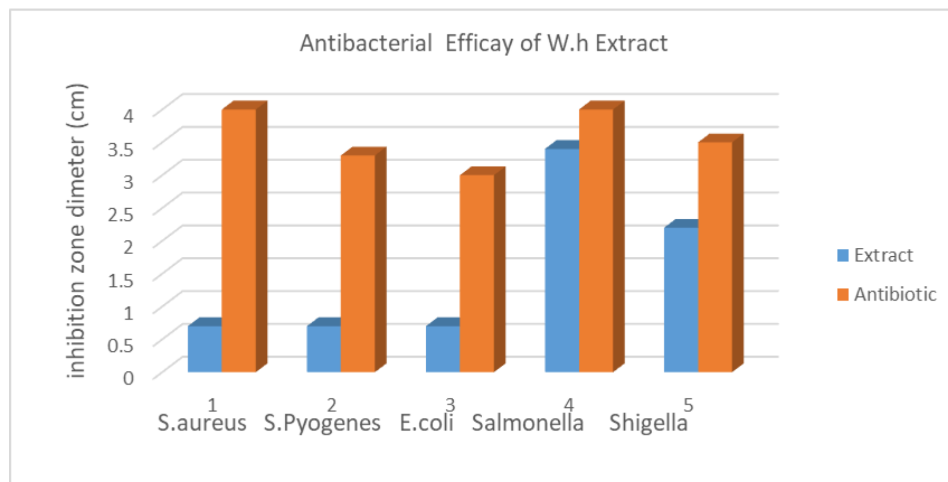

Figure (12): Inhibition Zone Diameters of Sage Extract with Hot Water Against Bacteria

The results demonstrated that the plant extract, prepared using cold methanol as a solvent, exhibited the greatest efficacy against S. aureus, with an inhibition zone diameter of 3 cm. The extract demonstrated an inhibitory effect on Salmonella, with an inhibition zone diameter of 2.2 cm. The inhibition zone diameter for Shigella was 1.8 cm, while that for S. pyogenes was 1 cm. The effect on E. coli was an inhibition diameter of approximately 0.65 cm, as illustrated in Figure 13.

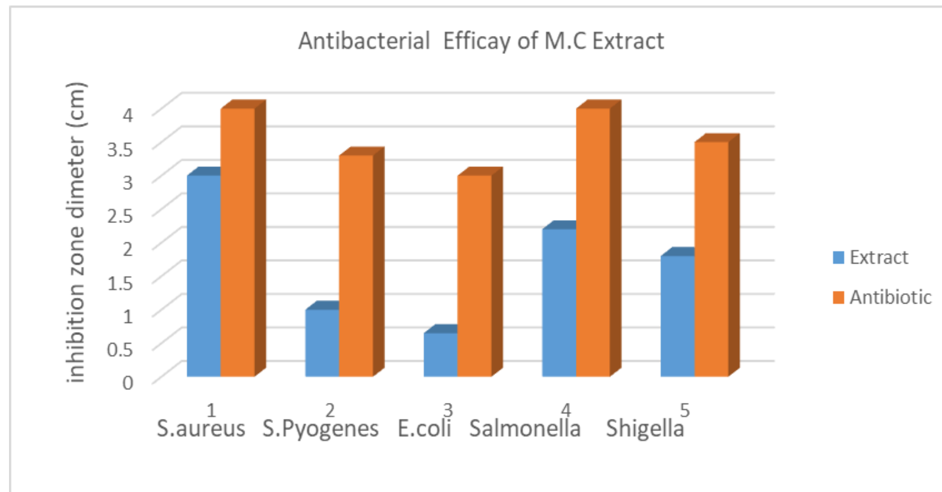

Figure (13): Inhibition Zones of Sage Extract Using Cold Methanol Against Bacteria

Conclusion

The consequences demonstrated that the aqueous and alcoholic extracts of sage, which are locally available in the suburbs of Sirte, displayed varying efficacy in opposition to various strains of pathogenic bacteria. Additionally, the findings demonstrated that the plant is rich in secondary metabolites, which play a pivotal role in the treatment of various illnesses. Based on the obtained results, it can be concluded that the locally available sage can be utilized as a safe antibacterial agent in the pharmaceutical and food industries.

Recommendations

It is recommended that the chemical content of the local sage and the efficacy of its essential oils be studied. Furthermore, a quantitative analytical study of the active compounds extracted from this plant by identifying their molecular structure is advised.

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