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ANTIBACTERIAL EFFECTS OF SELECTIVE SAUDI WILD MEDICINAL PLANTS

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ABSTRACT

Background: Medicinal flora is taken into consideration new sources for producing agents that might act as alternatives to antibiotics within the remedy of antibiotic-resistant bacteria. The aim of this observe was to estimate the antibacterial activity of the extraction for five plants against *Bacillus cereus* (*B. cereus*), *Streptococcus pneumonia* (*S. pneumoniae*) and *Staphylococcus aureus* (*S. aureus*) as a Gram-positive bacteria and *Escherichia coli* (*E. coli*) as a Gram-negative bacterium.

Methods: Experimental, *in vitro,* the activities of aerial parts of five ethanolic plant extracts as well as some antibiotics against *E. coli, B. cereus, S. aureus* and *S. pneumoniae* were evaluated. The activity against 4 isolates was determined by disc diffusion method at several concentrations (100, 50, 25 mg / ml).

Results: Plant extracts showed anti-bacterial activity, the results showed that *Pulicaria crispa* extract affected all bacterial strains examined and the effect was concentration dependent. On the other hand, *Teucrium polium* extract showed the lowest effect on bacteria used in the study. There were two promising plants, *Pulicaria crispa* and Salvia spinose, both had potential antibacterial effects against bacterial strains tested.

Conclusion: Among the studied plants, *Pulicaria crispa* and *Salvia spinose* showed promising potency and efficacy as natural antibacterial agent.

Keywords: Gram-negative bacteria, Gram-positivebacteria, Antibioticresistance, antibacterial, plant extract, Saudi Arabia, wild plants, medicinal plants.

INTRODUCTION

Plants products were employed in the treatment of infectious diseases many centuries earlier than scientific trails. Accordingly, they have contributed into an improvement of antimicrobial drugs. Therefore, A significant body of literatures were studied the action of plants on plants or animals pathogenic microorganisms. In 1943, Osborn added approximately 2300 plant species that have ability to inhibit bacterial growth **[1]**.

In addition, Nickell was presented 157 vascular plant families that have antimicrobial activitie [**2**]. Sehgal revised the higher plants and their effects against microbes **[3]**. Furthermore, antimicrobial activity of several plants was surveyed by Farnsworth **[4]**. In India, the biological activity of 880 plants was tested by a group of scientists **[5, 6, 7]**. Another investigation of 197 plant species showed antimicrobial activities by Stoessl **[8]**. Banerjee and Gupta presented the antimicrobial activity results from several plants **[9]**.

Recently, numerous human pathogenic bacteria have developed resistance to commonly used antibiotics, due to the indiscriminate use of antibiotics.

Furthermore, many antibiotics with different undesirable side effects have forced many scientists to investigate for new antimicrobial substances from various sources, e.g. microorganisms or medicinal plants.

Antibiotic resistance is now a large menace to public health worldwide. By finding and producing antibiotics which are new and more effective, scientists are trying to conquer this challenge **[10]**. For instance, some bacteria have the ability to resist an artificial antibiotic such as: *S. aureus*, one of the most common infectious pathogens in the world, and has become an alarming threat to health **[11, 12]**; and *E.coli* which caused various types of infection. Entero hemorrhagic *E. coli* can produce toxin-like shiga, hence known as a toxin producing type of shiga *E. coli* **[13]**. In addition, *B.cereus* causes two different types of intestinal diseases. One is diarrheal and the other is more likely to lead to nausea / vomiting. *B. cereus* was also involved in eye, respiratory and wound infections **[14]**. *S. Pneumoniae*, is a part of the normal micro-flora of the upper respiratory tract. It can become pathogenic under some conditions, as with many natural flora, usually when the host's immune system is weakened. All major virulence factors are invasions, such as pneumolysin, an antiphagocytic capsule, various adhesives, and immunogenic cell wall components **[15].**

Plants naturally produce a variety of compounds with known healing properties and anti-infective agents. These includes flavonoids, polyphenols and alkaloids. The screening of plants grown in Saudi Arabia, for antimicrobial activity showed that these plants or their extracts are potential sources for new antibiotics. *Salvia spinosa* L. (*Lamiaceae*, vernacular namelocal name: Athanalhumar) is a native plant in the Middle east, north Africa and east of Asia, is used in folk medicine as an anti-stomach disturbance, anti-inflammatory, anti-tussive, anti-rheumatic, carminative and hypotensive drug **[16]**. *Teucrium polium* L. (*Lamiaceae*, vernacular name: Jeaadah). Native plant in Morocco, Spain, France, Jordan and Saudi Arabia, used as an anticonvulsant, anti-inflammatory, analgesic, antipyretic, antimicrobial activity, antioxidant, anti-ulcer and can be used to heal wounds and lowering blood pressure **[17, 18]**. *Withania somnifera* (L.) Dunal (Solanaceae, vernacular name: Abab), which is generally native in Africa, Middle East and east of Asia is used in traditional medicine. Among its various physiological actions, its action on central nervous system primarily involves its effect against cognitive impairment, anxiety, and depression **[19]** due to its antioxidant, gamma-aminobutyric acid mimetic, and dopaminergic actions. It uses in Ayurvedic medicine, as a rejuvenator and as anti-aging compound **[19, 20]**. *Ephedra foeminea* Forssk. (Ephedraceae, vernacular name: Alandy) is a native in the Middle east, Algeria, Italy, Somalia and Turkey. Traditionally, it has been used to recover kidney problems like kidney stones. The stem of this plant is recognized to contain alkaloid ephedrine which is useful to treat many respiratory diseases such as asthma **[21, 22]**. *Pulicaria crispa* (Forssk.) Oliv. (Asteraceae, vernacular name: Gethgath) is worldwide plant, been used as galactagogues, antiepileptics, antimicrobial, antifungal and antioxidant due to presence of active phytochemicals **[23, 24]**. This plant also used to treat inflammation by the people of Saudi Arabia and Egypt, insect repellent and as an herbal tea **[25]**.

Considering the enormous potentiality of plants as sources for antimicrobial drugs, this study aimed to examine in vitro antibacterial activity of ethanolic extracts from five selected wild plants grown in Saudi Arabia against bacterial isolates.

3. MATERIAL AND METHODS:

3.1. Plant material

Five plants were gathered from Medina city, west-north of Saudi Arabia and used in this study. These plants were: *Salvia spinosa* L., *Teucrium polium* L., *Withania somnifera* (L.) Dunal, *Ephedra foeminea* Forssk. and *Pulicaria crispa* (Forssk.) Oliv. Plants were identified by Professor Samy Zalat, Biology Department, Faculty of Science, Taibah University. The areal parts of each plant were partially air dried for 4-5 days at room temperature. It was then sliced and ground before extracted using an ethanol-water mixture 70% (70/30V/V) for 48h. The concentrated ethanolic extract of each plant was filtered, then concentrated at a temperature below 50°C under reduced vacuum pressure. The obtained concentrate was kept at 4°C for later utilisation.

3.2. Phytochemical screening

The occurrence of secondary metabolites, such as saponins, tannins, flavonoids, coumarins, alkaloids, quinone, anthraquinones and terpenoids, in tested plants was illustrated based on previous studies **[26, 27]**.

3.3. Bacterial isolates

In this work, clinical bacteria isolates were kindly provided by Medina King Fahad hospital. They are three Gram-positive bacteria: *S. aureus, B. cereus* and *S. pneumoniae* and one Gram-negative: *E. coli*.

3.4 Antibacterial activity

The antibacterial activity of the selected plant extracts was assessed against the selected available microorganisms at different concentrations using a slightly modified agar disc diffusion process.

This experiment used three concentrations (100, 50 and 25 mg / ml) **[28, 29]**. As a standard, Ampicillin (Amp10), Erythromycin (E15), Rifampicin (RD5) and Clindamycin (DA2) were used for positive control. From extract, this gave 10 mg in10 ml of the test tube. Ten ml of dimethyl sulfoxide (DMSO) has been added to each extract and different substances prepared are marked according to their concentrations. The preparation of the 100, 50 and 25 mg / ml was accompanied by the serial dilution technique. A cotton bird was used to equally spread different bacterial isolates onto Müller Hinton agar Petri dish. Then, the filter paper (discs of 6 mm size) were loaded with each plant extract before placing on Müller Hinton agar. All plates were incubated for 24 hours at a temperature of 37ºC. After 24 hours of incubation, inhibition zones were measured by calculating the diameter of the growth inhibition zones in (cm) and determining antibacterial activities.

3.5 Statistical analysis

Data were expressed as mean ± SD using Graph Pad Prism software (version 5) and ANOVA followed by Tukey post-hoc test. $p<0.05$ was considered significant.

RESULTS

Phytochemical screening of five aerial plant extracts

Qualitative screening of the phytochemical extracts of five aerial plant extracts confirmed the incidence of saponins, alkaloids, tannins, flavonoids and quinones and the absence of triterpenes, anthraquinones, sterols and cardenolides. All phytochemicals are summarized in **Table 1**.

Table 1: The qualitative screening of phytochemicals of the five plants

Scientific name	Constitutes
Salvia spinose	1,8-cineol, (Z)-β-ocimene, germacrene d, 2-Butyl thiophene, trans caryophyllene and 3- Butyl thiophene [30].
Teucrium polium	neoclero-dane diterpenoids, sesquiterpenes, monoterpenes, polyphenols, flavonoids, fatty acid, terpenoids and iridoids [31, 32].
Withania somnifera	Phenols, flavonoids, tannins, saponins, alkaloids and cardic glycoside [33].
Ephedra foemina	flavonoids, tannins, terpenoids, phenols, carbohydrates, proteins & amino acids, reducing sugar, sterols and steroids, cardiac glycosides, diterpenes, lignin, saponin and flavanones [34].
Pulicaria crispa	alkaloids, saponins, proteins, tannins, phenols, flavonoids, cardiac glycosides, terpenoids (diterpenes, sesquiterpenes) [35].

Table 2: The antibacterial activities of five aerial plant extracts were examined against four standard bacterial isolates (Inhibition zones representing in centimeters)

Bacterial species / Positive control	*E. coli*			*S. pneumoniae*			*S. aureus*			*B.cereus*		
AMPICILLIN (Amp 10)	2.71 ±0.02			2.81 ±0.02			2.75 ±0.04			2.8 ±0.04		
ERYTHROMYCIN (E15)	2.51±0.02			2.48±0.02			2.51 ±0.02			2.46±0.02		
RIFAMPICIN (RD5)	2.78±0.02			2.73±0.02			2.71 ±0.02			2.81±0.02		
Plants												
Doses (mg/ml)	25	50	100	25	50	100	25	50	100	25	50	100
Salvia spinosa	--	--	1.78 ±0.06	2.3 ±0.04	2.48 ±0.06	2.65 ±0.04	--	1.75 ±0.04	2.03 ±0.04	2.83 ±0.04	2.93 ±0.04	3.03 ±0.04
Teucrium Polium	--	--	2.08 ±0.06	--	--	--	--	--	--	--	--	--
Withania somnifera	--	1.85±0.04	2.2 ±0.04	--	--	--	2.2 ±0.04	2.38 ±0.02	2.5 ±0.04	2.93 ±0.02	3.06 ±0.04	3.18 ±0.02
Ephedra foemina	--	--	--	--	--	--	--	--	--	2.8 ±0.04	2.95 ±0.04	--
Pulicaria crispa	2.03±0.04	2.35±0.04	2.5±0.04	2.16±0.04	2.28 ±0.02	2.51 ±0.02	2.2 ±0	2.35 ±0.04	2.56 ±0.04	2.91 ±0.02	3.06 ±0.04	3.18 ±0.02

Values are mean inhibition zone (cm) ± S.D, (--) bacteria were not affected by the plant extract

The Antibacterial activities of five aerial plant extracts were examined against four clinical bacterial isolates and at different concentrations as follows (25, 50 and 100 mg/ml) **Table 2**.

The results in **Table2** showed that the *Pulicaria crispa* effected all bacterial strain examined, whereas *Teucrium polium* was the lowest.

Pulicaria crispa was the most effective plant on all bacterial strains used. It effects *E. coli* in all concentrations and the effect was concentration dependent. Moreover, it effects *S. pneumoniae* and was dose concentration dependent. *P. crispa* effected *S. aureus* in all concentrations. The ethanolic extract of *P. crispa* effects *B. cereus* at all concentrations tested, and the effect was dose dependent.

S. spinose ethanolic extract effects *E. coli* at dose 100mg/ml, and effected *S. pneumonia*e at all concentrations, the effect was concentrations dependent. *S. spinose* effects on *S. aureus* and *B. cereus*, similarly, *S. pneumoniae*.

W. somnifera effect on *E. coli* was at both doses 50 and 100 mg/ ml. However, no effect on *S. pneumoniae*. Ethanolic extract of *W. somnifera* effects *S. aureus* at all doses and the effect was dose dependent, same effect on *B. cereus*.

E. foemina effect on *B. cereus* at 25, 50 mg/ ml only, and there were no effects on other bacteria.

T. polium effect on *E. coli* at dose 100 mg/ ml and no effect on other microorganisms.

DISCUSSION

Bacterial resistance to a large number of antibiotics is a serious global concern from a medical point of view. Hence, figuring out new chemical compounds affecting pathogens biologically is a key need to overcome harmful microorganisms. In this study, aerial parts of five Saudi (Medina city) wild plants were extracted in ethanol and examined against bacteria representing Gram-positive and Gram-negative.

Plants produce secondary metabolites with wide and variety productions, like coumarins, quinones, tannins, flavonoids, steroids, saponins, terpenoids, glycosides and alkaloids **[36]**. These biomolecules are the origin of plant-derived-antimicrobial-substances **[37]**. Several natural products have a strong effective in the bacterial infections treatment **[38]**.

In fact, the Gram-positive bacteria were more resistance to the extracts than the Gram-negative bacteria **(Tables 2)**.

However, data illustrated no statistically significant differences ($p > 0.05$). The differences showed in the current data could be due many factors. The extraction method with 70% ethanol might give different results as compared to other studies. The outcomes are harmonizing with previous compounds findings from *Ochna* species isolations **[39]**. In addition, the difference in the cell wall structure could be the reason of sensitive distinction between Gram-positive and Gram-negative bacteria. Peptidoglycans are 70-100 layers in the cell wall of Gram-positive bacteria and comprised of two polysaccharides types, *N*-acetyl-muramic acid and *N*-acetyl-glucosamine cross linked by cross bridges and peptide side chains. This is an over simplification of the possible role of cell wall structure of different bacteria strains that may explain their different sensitivity plants extracts. The Gram-negative bacteria can resist some of antibiotics such as penicillin by the lactamase enzyme secretion which exist in the periplasmic space between the cytoplasmic membrane and the thin outer membrane **[40]**.

The correlation between flavonoids and antibacterial activities has been reported previously. In fact, the lipophilic properties of these substances enable them to deteriorate the cell wall and cell membrane of microorganisms, to inhibit nucleic acid synthesis, structural and enzymatic proteins **[41]**. They have the capacity to form complexes with extracellular and soluble proteins and with the cell wall **[42]**.

CONCLUSION

In this work, the antimicrobial activity of ethanolic aerial parts extracts of different Saudi wild plants was evaluated against Gram-positive and Gram-negative bacteria. The results of this study indicated that the ethanol extracts were effective as promising antibacterial agents. The antimicrobial activity of *Pulicaria crispa* and *Salvia spinose* extracts against bacteria was more effective than other extracts. Further work needs to be done with more microorganism's species. Identification of the active compound in these plants are also required to assess their role as antimicrobial drug. the extracts efficacy requires further studies to examine possible inhibition mechanisms occurred by the five plant extracts. Interestingly, it also could study the extraction action modes against resistant of these clinical isolates and other pathogenic bacteria.

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