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Evaluation of the Biological Activity of Some Plant Extracts Against Gram-Positive and Gram-negative Bacteria

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Abstract. *The inhibitory activity of two types of plant extracts, namely the alcoholic and aqueous extracts of Capparis and Artemisia, on the growth of two types of biologically important bacteria included Bacillus cereus, a Gram-positive bacterium, and Pseudomonas aeruginosa, a Gram-negative bacterium, one of the most important opportunistic pathogens.The plant extracts were prepared at different concentrations (10, 20, and 30 mg/ml), and their effect was tested using the diffusion method in the Mueller-Hinton medium. The results showed that the alcoholic extracts were more effective in inhibiting the growth of both bacteria types than the aqueous extracts. The study demonstrated that plant extracts containing active chemical compounds, particularly alcoholic ones, could be used as effective natural antibiotics in healthcare. To pave the way for further comprehensive studies to identify these compounds, their effects, and their potential benefits. These results may contribute to the development of natural therapeutic alternatives to combat bacterial infections or be used to indicate the effectiveness of active chemical compounds present in plants by inhibiting B. cereus.*

Keywords: *Biological activity, plant extracts, P. aeruginosa and B. cereus*

INTRODUCTION

Plant extracts are widely used in traditional medicine [1, 2] because they contain thousands of active chemical compounds with antimicrobial activity against many pathogens [3], making them promising natural alternatives to pharmaceuticals, food preservation, and alternative energy. These extracts are also relatively safe and harmless, and have fewer side effects than antibiotics and synthetic substances, enhancing their value in therapeutic and preventive applications in [4]. Accordingly, two active plant species, wormwood (*Artemisia* spp.) and caper (*Capparis spinosa*), were selected, and the effect of their alcoholic and aqueous extracts on the growth of two Gram-positive and Gram-negative bacteria was evaluated. *Artemisia* is a perennial plant with threadlike, branching roots [5]. Numerous active chemical compounds, including more than 59 aromatic compounds, have been identified, most notably artemisinin and sesquiterpene lactone [6], which exhibit distinct pharmacological activity against both Gram-positive and Gram-negative bacteria. These bioactive compounds have made *Artemisia* an important source of antimicrobial therapies, particularly in light of the increasing resistance of bacteria to

traditional medicines [7, 8]. Caper, a perennial thorny shrub with deep roots and a widespread distribution, is a rich source of bioactive compounds with potential nutritional and medicinal applications [9]. Among the active compounds extracted from it are glucocaprin, rutin, spermidine, quercetin, kaempferol, stigmasterol, campesterol, tocopherol, and carotenoids, in addition to a group of antioxidants with protective effects, including their role in protecting the liver and tissues from oxidative damage [10].

Meanwhile, *Pseudomonas aeruginosa* is one of the most common Gram-negative pathogens in clinical settings and is associated with hospital-acquired infections and increased antibiotic resistance. According to the National Institutes of Health in the United States, more than 80% of microbial infections depend on biofilms formed by this bacterium, making its study essential for developing new therapeutic strategies [11]. Bacillus cereus it is a Gram-positive bacterium, a common bacterium that causes food poisoning; It is the third most common cause of food poisoning [12]. It is also an important biological model in evaluating the effectiveness of active compounds extracted from plants, which helps measure their effect on other, more resistant bacterial strains [13].

METHODS AND MATERIALS

A. Sample collection and identification

Samples of *Artemisia* spp were collected from local markets in (500 g), where the plant type was identified and its identity confirmed by specialists to ensure accurate classification. The Capparis samples Capparis spinosa were collected directly from natural environments in Thi-Qar Province, and the aerial green parts were selected for use in the study.

After collecting the samples, they underwent a thorough cleaning process that included washing with distilled water to remove dust and suspended impurities; then, they were left to dry at room temperature away from direct sunlight to preserve the biologically active compounds. After drying, the samples were ground using an electric mixer until a fine powder was obtained. Then, the powder was stored in dark, tightly sealed containers away from light and moisture sources to ensure the stability of the chemically active materials until they were used in laboratory experiments.

B. Preparation of alcoholic and aqueous extracts

30g of the previously prepared plant powder was dissolved in (150 ml) of ethanol at a concentration of (80%), and the mixture was kept in the laboratory room for (48 hours); then, the mixture was filtered using several layers of medical gauze to remove large insoluble particles. After that, the mixture was centrifuged at a speed of (5000 rpm for 15 minutes), then the precipitate was removed, and the remaining suspension was dried, collected, and kept in the refrigerator at (4 °C) until use.

The dry extract was dissolved in (10%) dimethyl sulfoxide (DMSO), and then the concentrations (100 mg/ml, 200 mg/ml, and 300 mg/ml) were prepared, respectively, and kept until use at a temperature of (4 °C). The same previous method was used: the powder

was dissolved in (150 ml) of distilled deionized water using a magnetic stirrer, and then the method was completed [9, 14].

Before starting the experiment, the dried plant extract powder was dissolved in sterile distilled water to obtain a final concentration (100 *mg/ml*, 200 *mg/ml*, and 300 *mg/ml*) and stored at 4°C.

C. Isolation and identification of bacteria

The bacteria used in the study were isolated in the microbiology laboratory of the College of Applied Medical Sciences at Shahrah University using morphological, microscopic, and biochemical diagnostic methods. blood-agar and Mueller-Hinton culture media were used to grow the isolates. Then, further serial dilution was done by adding 1mL of the aliquot into (9 *mL*) of sterile distilled water to make an appropriate dilution (10^{-5} – 10^{-6}) before growing them on Mueller-Hinton culture.

D. Antibacterial activity test of plant crude extracts

Effective bacterial colonies were isolated from blood-agar medium and transferred to test tubes containing appropriate growth medium.

A decimal dilution was performed to reach a concentration of (10−5 – 10−6). The bacterial dilutions were then cultured on Müller-Hinton Agar medium using the Spread Plate Method to ensure a homogeneous distribution of bacterial cells on the surface of the medium.

The inhibition assay was performed by making wells in the culture medium, where (100 µl) of the diluted bacterial suspension was added to each well. The plates were left for (30 min) at room temperature to ensure the spread of the bacterial suspension and saturation of the medium and then incubated at (37 °C for 20 –24 h). After the incubation period, the diameters of the inhibition zones were measured using a millimeter ruler, and the results were recorded accurately according to the standards adopted in antibiotic susceptibility testing [15].

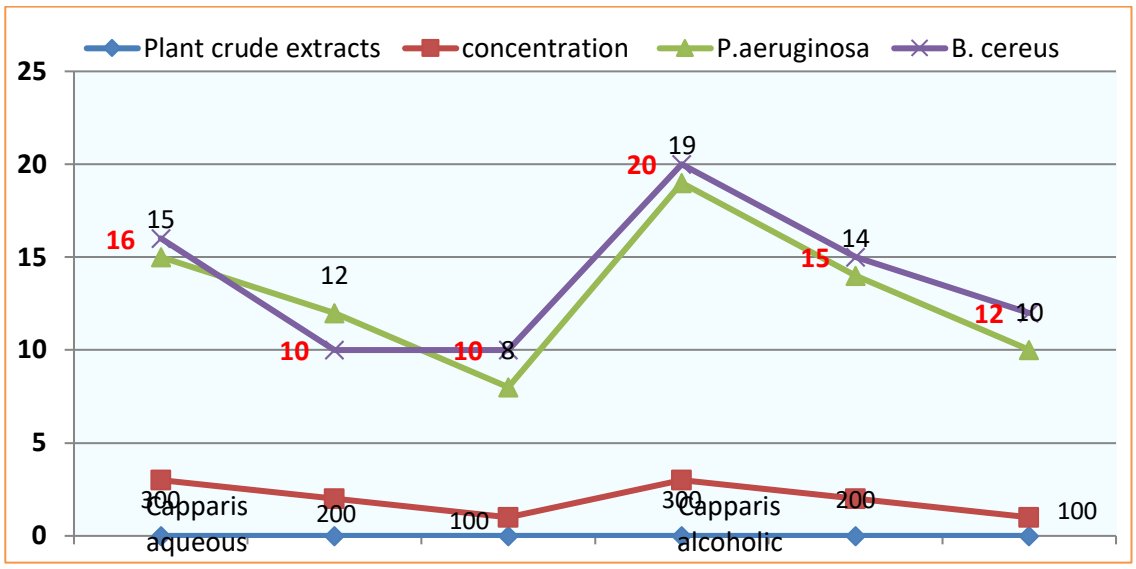
RESULTS AND DISCUSSION

A. Result

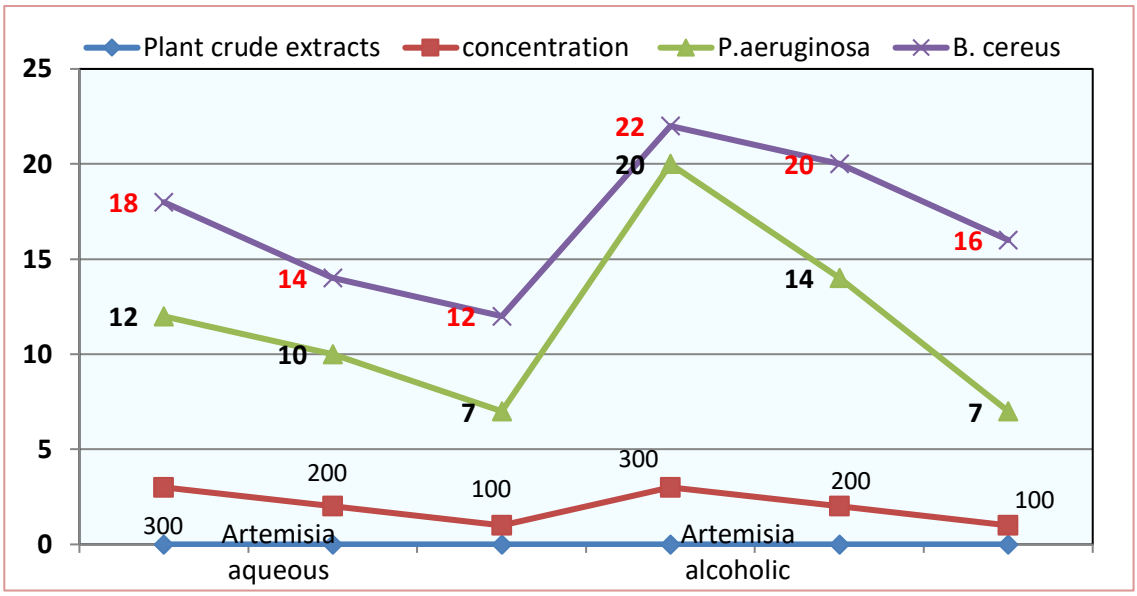
The current results, shown in Figures 1, 2 and 3 demonstrate the biological activity of alcoholic, methanolic, and aqueous plant extracts of some vegetative parts of *Artemisia* and Capparis spinosa plants against two bacterial strains, one Gram-positive and the other Gram-negative, using the traditional disk diffusion method on Mueller-Hinton medium.

At a concentration of (300 *mg/ml*), the alcoholic and methanolic extracts of both plants demonstrated a biological activity that warrants further close monitoring. The inhibition diameters for both isolates were (*7 mm - 22 mm*), while the aqueous plant extracts showed lower inhibition rates than the alcoholic extracts, which showed an inhibition rate of *(7 mm - 16 mm)*. These results varied in terms of inhibitory activity when using three different

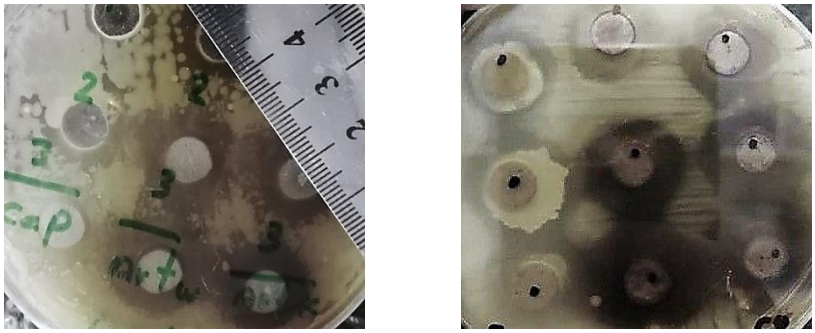
concentrations and several iterations of the plant extracts, with activity increasing with increasing concentration and at a specific concentration. Three replicates were used for each concentration. To ensure the accuracy and reliability of the results, three replicates were used for each concentration, which strengthened the validity of the data and confirmed the gradual effect of the extracts on bacterial growth. These results were similar to a previous study in which caparison roots were used instead of plant parts, suggesting that alcoholic extracts of plant parts could be an effective alternative for their antibacterial effect, as shown in the following figures (1, 2, 3).



Figures 1. Effect of alcoholic and aqueous *Capparis* extract



Figures 1. Effect of alcoholic and aqueous *Artemisia* extract



Figures 3. Zone of inhibition on Mueller-Hinton culture of isolates

B. Discussion

Ethanolic and aqueous extracts of the green parts of *Capparis spinose*, showed clear effectiveness against the bacterial isolates used. Their effect on *P. aeruginosa* was similar to what was reported in a previous study that used *Capparis* root extracts [16], indicating the effectiveness of the chemical compounds present in the different parts of the plant [15]. The results were also consistent with what was reported in study of Gull *et al*., [17], with a relatively lower response observed for *P. aeruginosa* bacteria. This variation in response may be attributed to the nature of the cell wall of Gram-negative bacteria, as it contains an outer layer of phospholipids that limit the permeability of active compounds compared to Gram-positive bacteria, which have a more porous layer of peptidoglycan, making them more susceptible to plant extracts [18].

The results of the aqueous extracts were consistent with study of Iqbal *et al*. [19], and study of Arangale *et al*. [20], indicating a similar effect pattern when using aqueous solvents. This difference between the extracts is likely due to the nature of the solvent used in the extraction process, as solvents differ in their polarity and chemical properties, which affects the extraction of biologically active compounds, as indicated by study of Sultana *et al*. [21], as for the extract of *Artemisia*, whether alcoholic (Ethanolic) or aqueous, the results showed positive efficacy consistent with what was reported in previous studies [22, 23].

The effect of the plant extract activity on the bacterial isolates was determined based on the formation of the inhibition zone and its diameter in mm [15].

Confirming the possibility of benefiting from these extracts as natural antimicrobials with a broad-spectrum effect.

CONCLUSIONS

The study confirmed the effectiveness of alcoholic (Ethanolic and Methanolic) and aqueous extracts in inhibiting the growth of the tested bacterial isolates, which enhances their potential use as natural sources of compounds with antibacterial properties. The results showed that alcoholic extracts were superior to aqueous extracts in inhibitory activity, indicating that the active antibacterial compounds in these plants dissolve better in organic solvents. The study showed that the vegetative parts of the *Capparis* plant have an effectiveness similar to what was recorded in previous studies when using the roots, which indicates that the active compounds may be distributed in different parts of the plant.

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Conflict of Interest Statement:*The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.*