PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *Capparis spinosa* ROOTS EXTRACTS AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT

Medicinal plant is one of the most effective and safe for the treatment of human diseases. Extract from root of *Capparis spinosa* was prepared in petroleum ether, ethyl acetate and methanol by employing three different methods of extraction viz. Ultrasonication, Soxhlet methods and extraction by continuous shaking at room temperature. The phytochemical results revealed different components of plant extracts with the different solvents based on the polarity of each solvent. The results show that there is significant difference among all three studied solvents for each extraction method at p ≤0.05, since p values are 0.000, 0.000 and 0.001 respectively. However, there is significant difference among the three different studied extraction methods since p ≤ 0.05, for the two solvents, (petroleum ether and methanol), wherep values are 0.040, 0.001 respectively. While, for ethyl acetate, there is no significant difference. The minimum inhibitory concentration (MIC), of crude extracts, by three solvents (Petroleum ether, Ethyl acetate and methanol) against *S. Epidermidis* were 50 mg/ml for petroleum ether extract and 200 mg/ml for the remains. The MIC was the same for the three solvents extracts against *S. aureus* was 200 mg/ml. Finally, the MIC was 200 mg/ml for petroleum ether extracts and 100 mg/ml for ethyl acetate and the methanol extracts against *E. coli*.

Keywords:*Capparis spinosa*, root, antibacterial, pathogenic bacteria

INTRODUCTION

From a while, one of the most significant effect for therapeutic and treatment of human diseases safely are medicinal plants [1]. These medicinal plants have a numerous health-promoting effects to save human life [2]. As it is known that the synthetic drugs may cause a wide range of serious effects on human lives [3]. For this reason, the researchers should concentrate their thinking about the roles and beneficial effects of medicinal plants, which possess a safely usage in the therapy of human diseases [4].

Recently, phenols and flavonoids are considered as important bioactive natural products extracted from the medicinal plants [5] in addition to another herbal formulatesthathavemany beneficial roles for human health[6]. This made them as important alternative therapeutic agents of different human diseases such
as cardiovascular diseases, neurodegenerative disease since the medicinal plants have high efficiency and low adverse effects [7-9].

Caper (Capparisspinosa L.) is a main member of the genus Capparis (Capparidaceae family) [9]. The Caper is an endemically wood shrub, prickly, 0.3–1m tall, and is commonly known by different names such as Caper (English), Alcaparro (Spanish) [9], Alaf-e-Mar (Persian) [10] and Cappero (Italian) [12].

The roots of Capparisspinosa L. have been extended up to 6-10 m. It is widely presented in the worldwide extended from Morocco to Crimea, Armenia and Iran [10,13]. C. spinosa L. has a wide bactericidal effect against gram-positive and negative-bacteria. In other words, it appeared important effect against biotic and abiotic stresses [10].

Last two decades, C. spinosa shown pharmacological effects because of containing numerous bioactive constituents in particularly its polyphenolic compounds. It is a rich source of polyphenols and active constituents which made researchers focused on their health-promoting effects to save human life [14]. Moreover, C. spinosa L. possesses antioxidant, antimicrobial, anticancer and hepatoprotective effects, which resulted in its gaining different pharmacological effects [15]. In traditional medicine, varying parts of C. spinosa have been widely used for the treatment of various human diseases [16] such as aerial parts and roots that have been used for the treatment of rheumatism, gastrointestinal problems, headache, kidney and liver disease as well as toothache [17]. Arabian traditional medicine has been proposed to use leaves, roots and buds of C. spinosa L. in the treatment of spleen diseases, stomach problems, skin diseases, earache and kidney diseases as well as hepatic diseases in addition to the recommendation for the treatment of paralysis, convulsions and gum problems [10]. Iranian traditional medicine suggested that C. spinosa roots, fruit and bark have been used as diuretic, tonic and antimalarial agents [18, 19]. Leaves of C. spinosa have been used as antibacterial activity against pathogenic bacteria [19] in addition to its beneficial effects on coughs and asthma [21].

This paper aims to show the Capparis spinosa L. root extractions by three different solvents in three different methods of each solvent at room temperature.

MATERIALS AND METHODS

Plant material

Capparisspinosaroot were collected from Samawa city during the July 2017. The plant identified by a botanist at Al Muthanna University. The plant roots washed and dried at room temperature in the dark for 14 days and then finely ground by using an electric grinder.

Microbiological material

The bacterial strains were used responsible for various infections such as skin and urinary tract which are a major problem for public health are Gram-Positive and negative Bacteria (Staphylococcus aureus, Staphylococcus epidermidis and E- coli). These strains provided by the bacteriology laboratory of Al Hussein Teaching Hospital. These bacteria were isolated from patients and were maintained by subculture on agar media.

Extraction of Bioactive Compounds
Root was extracted through maceration, Soxhlet and ultrasonic methods, separately, using three selected solvents (Methanol, Ethyl acetate, and Petroleum ether).

**Ultrasound-Assisted Extraction**

*Capparis spinosa* root (10 g) and 100 ml of each solvents are introduced into a flask. The mixture was exposed to bath ultrasound for 1 hour under 60 kHz at room temperature and sheltered from light. The later process has been repeated under same condition for ten times. Afterward, the mixture was filtrated and the final volume (accumulate filtrate) is concentrated in rotary evaporator under reduced pressure\(^1\).

**Soxhlet Extraction**

About 10 g of plant sample (powder) was placed in thimble and extracted with selected solvent, separately, in Soxhlet extraction unit (MAC). Extraction was repeated until the sample extract became of color less\(^2\).

**Magnetic Stirrer Extraction**

About 10 g of plant sample (root separately) was mixed in different solvents, separately, in a ratio of 1:10 (dry powder: solvents). The mouth of conical flask was sealed with the para-film. Samples were macerated using rotary shaker at 3000 rpm for 24 h\(^3\).

**Preliminary Phytochemical Screening**

The condensed extracts are used for preliminary screening of phytochemicals such as alkaloids, flavonoids, steroids, terpenoids, tannins, saponins, carbohydrates, glycosides, proteins, amino acid according to\(^{4-16}\).

**Antimicrobial susceptibility testing**

The crude extracts were dissolved in 10% DMSO, then they were twofold serially diluted in a liquid growth medium (MHB) containing 0.78 – 400 mg/ml of each extract. After shaking, 100 μl of each extract dilution was added to each well of 96 well micro titer plates, except the 12th well (growth control, without the crude extracts). Then, each well is inoculated with 100 μl of a microbial inoculum (1×10^6 CFU/ml), except of the 11th well (sterility control, without bacteria), and incubated at 37±2°C. MIC was measured at 620 nm. The plate was measured pre- and post-incubation at 37°C for 24 hours. According to\(^{17}\), calculation of the Bacterial growth inhibition was achieved by the following equation\(^1\).

\[
\text{Percentage growth inhibition} = \left( \frac{\text{OD of control}}{\text{OD of control} - \text{OD of test}} \right) \times 100
\]

**Statistical analysis**

This study designed by Completely randomized design (CRD) that used in the statistically analysis of variance for data of plant extraction yields and the crude of plant activity against bacteria by using one-way ANOVA test, independent t-test and Dunnett's test at a 5% level of significance. Data were processed and analyzed by using statistical program social science (SPSS 22) and the results were expressed as Mean±SD \(^{18}\).

**RESULTS AND DISCUSSION**

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Yield of Crude Extraction

In this study, root extraction yields are statistically analyzed to find the significant differences among both solvent types and extraction methods. The results show that there is a significant difference among all the three studied solvents (Petroleum ether, Ethyl acetate and Methanol) for each extraction method (Magnetic Stirrer, Soxhlet Extraction and Ultrasonic) at p≤0.05, where p values are 0.000, 0.000, 0.001, respectively, that is shown in Table (1). The methanol has the highest yield and followed by ethyl acetate but the petroleum ether extracts have the lesser yield in comparison to both other solvents and this comes in agreement with others studies [19,21]. In addition Kwei has reported that extraction using a higher polar solvent gives better yield [22].

Table 1: Comparison of root extraction yields among both solvent types and Extraction Methods.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Extraction Methods</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Magnetic Stirrer</td>
<td>Soxhlet Extraction</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>0.239±0.06</td>
<td>0.348±0.09</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.83±0.23</td>
<td>0.993±0.25</td>
</tr>
<tr>
<td>Methanol</td>
<td>8.89±1.8</td>
<td>24.58±4.3</td>
</tr>
</tbody>
</table>

* represent a significant difference at p≤0.05, letters (a and b) represent type of the statistical analysis: a- among solvents types, b- among root extraction methods.

In addition, there is significant difference among the three studied of extraction methods (Magnetic Stirrer, Soxhlet Extraction and Ultrasonic) p<0.05, for two solvents (Petroleum ether and Methanol), where p values are 0.040, 0.001, respectively, but there is no significant difference among the three studied of extraction methods for ethyl acetate solvent. This depends on the fiber composition of the roots, the nature of the binding of the active compounds to them and the polarity of the solvent.

The ultrasonic method is the best techniques to extract bioactive compounds from the root of *C. spinosa* due to the Ultrasonic method that requires less time in comparison to others method in the study. In addition, the sonication-assisted extraction is carried out at a lower temperature compared with the Soxhlet technique, which is favorable for the thermally unstable compounds according to [23].

Phytochemical Composition of *Capparis Spinosa* roots

The Plant extracts have one of the most important sources of antimicrobial compounds especially against pathogenic organism [4].

Table 2: Phytochemical Analysis of Root extract.

<table>
<thead>
<tr>
<th>N</th>
<th>Name of test</th>
<th>Root</th>
</tr>
</thead>
</table>

Regarding preliminary phytochemical analysis of root extract, the results are tabulated in Table (2) showing the presence of various bioactive compounds in all the Solvents extracts studied. The extract from *C. Spinosa* roots viz the methanol as high polarity solvent shows that the roots extracts contained pharmacologically active components as alkaloids, flavonoids, steroids, Terpenoids, tannins, saponins, carbohydrate glycoside, protein, and amino acid, some bioactive compounds which may be responsible for the antimicrobial activity. In agreement with another study \[5\].

Ethyl acetate extracts has less polarity comparing with methanol. The bioactive compounds extract decrease with a decrease in polarity. In the same Table (2) shows that the root extracts contain the steroids, terpenoids, tannins, saponins, carbohydrates, and proteins with no of alkaloids, flavonoid and amino acid which are found in methanol extracts for comparison. But the petroleum ether is lowest polarities compared to other solvents are studied. The root extracts contain the steroids, terpenoids, saponins, carbohydrates, glycosides and protein, with no remained bioactive compounds are found in other extracts that studied.

**Antibacterial Activity of crude extract of root on pathogenic bacteria**

The crude extracts of plant by three different solvents (petroleum ether, Ethyl acetate and methanol) were evaluated for their antibacterial activity against three of the pathogenic bacteria by using a spectrophotometric assay as shown. These crude extracts show a significant difference at \(p \leq 0.05\) between Capparis spinosa extract concentrations and control for all the bacteria studied. The minimum inhibitory

<table>
<thead>
<tr>
<th></th>
<th>Petroleum ether</th>
<th>Ethyl acetate</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>4</td>
<td>Terpenoids</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Carbohydrates</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Proteins</td>
<td>++</td>
<td>+ +</td>
</tr>
<tr>
<td>10</td>
<td>Amino acid</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Symbols (-, +,++,+++)) represent the following: (-)= negative, (+)= few concentration,(++)= moderate concentration, (+++)= high concentration.
concentrations (MIC) of crude extracts by three solvents (petroleum ether, ethyl acetate and methanol) against *S. Epidermidis* were 50mg/ml for petroleum ether and 200mg/ml for ethyl acetate and methanol extracts respectively as shown in fig (1).

Figure 1: Growth inhibition of *Staphylococcus Epidermidis* caused by different concentration of the Root extract of *Capparis spinosa* for three different solvents. * represent a significant difference at p≤0.05 between *Capparis spinosa* extract concentrations and control. Data represent the mean ± SE.
Figure 2: Growth inhibition of *Staphylococcus aureus* caused by different concentration of the Root extract of *Capparis spinosa* for three different solvents. * represent a significant difference at p≤0.05 between *Capparis spinosa* extract concentrations and control. Data represent the mean ± SE.

![Growth inhibition of Staphylococcus aureus](image)

Figure 3: Growth inhibition of *E. coli* caused by different concentration of the Root extract of *Capparis spinosa* for three different solvents. * represent a significant difference at p≤0.05 between *Capparis spinosa* extract concentrations and control. Data represent the mean ± SE.

In addition, the MIC of crude extracts by same solvents against *S. aureus* was 200 mg/ml as shown in Fig.(2). Finally, the MIC of crude extracts by same solvents against *E. Coli* were 100 mg/ml for ethyl acetate and methanol extracts and 200 mg/ml for petroleum ether extract respectively as shown in Fig. (3).This result agrees with other studies [24,25]. The bacteria growth increases with a decrease in the concentration of extracts gradually. The petroleum ether extracts are effective to low concentration (6.25 mg/ml) on *S. Epidermidis*. This effect is due to the petroleum root extracts contains the nonpolar compounds in its constituents, such as fatty acids or its derivatives and steroids compounds that have antibacterial activity against a broad range of gram-positive and negative bacteria [26-29]. The growth inhibition concentration by ethyl acetate extracts is less compared with petroleum ether extracts in low concentration on *S. epidermidis*, but is more on *S. aureus* and *E. coli* than petroleum ether extract that may be depend upon the structure of bioactive compounds and the properties of bacteria. The activity of methanol extracts on bacteria is lower effect than the activity of the petroleum ether and ethyl acetate extracts on all bacteria. These results may depend on the chemical constituents that are found in crude extracts and its concentration or occur synergistically effect between these constituents. In other means, due to the presence of a functional group such as OH, and carbonyl, the compounds have antibacterial activity [30].

**CONCLUSION**

The study showed that Capparis spinosa leaves which were collected from As Samawah city, were found to be suitable for separation some compounds. The two separated compounds were showed antibacterial activity, so it might be using these compounds as an antibacterial drug with a low cost and available source.

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ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES


