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Evaluation of Antibacterial Activity of Selected Libyan Medicinal Plants against *Staphylococcus aureus* and *Bacillus subtilis*

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Abstract

Medicinal plants could represent an alternative promising antimicrobial agents. The aim of this study was to evaluate the antibacterial activity of *Zizyphus Vulgaris, Laurus nobilis, Thymus capitatus, Cistus salvifolius, Arbutus pavarii, Rhus tripartata* and *Pistacia atlantica* against reference strains of *Staphyloccocus aureus* and *Bacillus subtilis.* For this purpose, extract of leaves was obtained by 96% chloroform, 95% ethanol or petroleum ether. Antibacterial activity and minimum inhibitory concentration (MIC) for different extracts of each plant was evaluated. *Zizyphus Vulgaris, Cistus salvifolius, Thymus capitatus* and *Arbutus pavarii* were the most effective, as the values of inhibition zones were 20 ±2.5 mm, 18 ±2.1mm, 14 ±2.2mm and 15 ±2.0mm for *Staphyloccocus aureus,* and 10 ±2.1, 14.0 ±1.9, 15 ±2.0, and 15 ±1.7mm for *Bacillus subtilis,* respectively. However, *Pistacia atlantica* and *Laurus nobilis* were less effective against *Staphyloccocus aureus* and *Pistacia atlantica* and *Rhus tripartata* were less effective against *Bacillus subtilis. Cistus salvifolius* extract caused an inhibition of *Staphyloccocus aureus* growth at 25 mg/ml while the extract of *Thymus capitatus, Arbutus pavarii, Rhus tripartata* and *Pistacia atlantica* caused an inhibition at 50 mg/ml. However, the minimal inhibitory concentration was 200mg/ml for other plant extracts. The minimal inhibitory concentration for *Bacillus subtilis* was 50 mg/ml for *Thymus capitatus, Arbutus pavarii* and *Pistacia atlantica* extract, 100 mg/ml for *Cistus salvifolius* and 200 mg/ml for other tested plant extracts. Petroleum ether extract of *Zizyphus Vulgaris* was the most effective causing an inhibition zone of 14mm on *Staphyloccocus aureus* growth. However, petroleum ether extract of *Cistus salvifolius* was the most effective antimicrobial agent against *B. subtilis* growth. *Zizyphus Vulgaris* extract was effective at 100 mg/ml, and *Cistus salvifolius* was effective at 200 mg/ml. Chloroform extract of *Rhus tripartata* and *Thymus capitatus* were effective against *Staphyloccocus aureus* at 100 and 200 mg/ml respectively. The results of the present study indicate that ethanolic extract of *Cistus salvifolius, Arbutus pavarii* and *Rhus tripartata* has an effective and potent antibacterial activity.

Keywords: Medicinal plants, Extract, Antibacterial activity, Libya

1. Introduction

In developing countries, synthetic drugs used currently are expensive and inadequate for the treatment of diseases [1]. The development of antimicrobial resistance by microorganisms has increased. In general, bacteria have the genetic ability to acquire and transmit resistance to drugs, which are utilized as therapeutic agents [2]. Therefore, alternative antimicrobial agents of herbal origin may be of interest.

Staphyloccocus aureus and *Bacillus subtilis* cause a wide range of diseases in both human and animals [3, 4]. Development of antimicrobial resistance in bacteria has been documented extensively and has become a common finding [5, 6]. Recently, an extensive work has been done to examine the *in vitro* antimicrobial effects of numerous herbal plants [7, 8]. Plant extracts may include roots, stem, leaves or flowers [7, 9]. Additional antioxidant and food preservative effect of herbal plant extracts have also been documented [9]. The extraction of herbal plants is carried out by different methods and chemicals [10]. Several studies reported that extract of *Laurus nobilis* leaves has not only antibacterial and antioxidant effects [10, 11] but also has an antiproliferative activity against human breast adenocarcinoma cells [12]. *Rhus tripartitum* has been found to have antioxidant and antifungal activity [13, 14]. Polymers from *Pistacia atlantica* were screened against *Helicobacter pylori* and other Gram-negative and Gram-positive bacteria to evaluate their antimicrobial action [15]. However, a study on *Zizyphus spp.* documented its antimicrobial activity [16]. To the best of the author's knowledge, much less attention, however, has been given to the studies on the antibacterial effect of medicinal plants in Libya. Therefore, the objective of the present study was to assess the antibacterial effect of the ethanolic and ether extracts of seven Libyan medicinal plants against *Staphyloccocus aureus* and *Bacillus subtilis.*

2. Materials and Methods

2.1. Medicinal plants

Seven medicinal plants, namely, *Zizyphus Vulgaris, Laurus nobilis, Thymus capitatus, Cistus salvifolius, Arbutus pavarii, Rhus tripartata,* and *Pistacia atlantica* were randomly selected and collected from eastern regions of Libya.

2.2. Preparation of the plant material

The collected plant samples were cleaned using tap water to remove the dusts and then dried in an oven at 60 °C for 8 hr.

2.3. Preparation of different plant extracts

Extractions procedures were carried out at the Medicinal Plants Laboratory at Biotechnology Research Centre, Tripoli. Leaves of plant samples were separately grounded in a blender. Ten grams of each grounded tested plant were separately dissolved in a flask containing 100 ml of either 96% chloroform, 95% ethanol or petroleum ether for 72 hr. The samples were filtrated using Whatman No1 filter paper and the residue was re-extracted with the same used solvent. The filtrates were collected together and evaporated to dryness at 40 °C under reduced pressure. The residue was kept in the refrigerator at 4 °C until use according to previously described methods [17].

2.4. Bacterial isolates

Bacterial isolates, *Staphyloccocus aureus* (ATTC103307) and *Bacillus subtilis* (ATTC6633), were obtained from the Department of Microbiology at the Biotechnology Research Center. The bacterial isolates were grown on nutrient agar slants for 24 hr at 37 °C then used in the experiment.

2.5. Antibacterial activity assay

The antibacterial activity of different extracts (Ethanol, petroleum ether and chloroform) was evaluated by disc diffusion method [18]. Briefly, this method is based on the diffusion of an antibiotic from a filter paper disc through the solidified culture medium (Muller- Hinton agar) of Petri dishes. Whatman filter paper (No2) was used to prepare small discs (6 mm in diameter), then sterilized at 121 °C for 15 min in an autoclave. The discs were separately impregnated with the previous extracts for 1-4 hr at room temperature and allowed to dry. A swap of bacterial broth (previously prepared and adjusted to contain 108 cfu was dispersed on the agar plate surface. After drying, the previous discs were placed on the Petri dishes and then incubated at 37 °C for 24 hr. Three replicated plates were used for each treatment. The diameter of inhibition zone created by each disc was measured (in mm) using a ruler.

2.6. Determination of the minimum inhibitory concentration (MIC)

The MIC of the plant extracts was estimated on solid medium (nutrient agar) using the previously described method [19]**.** Six concentrations, i.e. 6.25, 12.5, 25, 50, 100 and 200 mg/ml were tested. Briefly, a stock solution was prepared by dissolving 200 mg of each extract in one milliliter of the solvent containing dimethylsulfoxide and water in a ratio of 2:4 v/v, respectively.

One hundred microliter of nutrient broth medium was dispensed into one well for each treatment to be a first control. The second control consists of other well contained only 100 µl of the extract. Another 100 µl of stock solution was transferred to a third well. A serial dilutions were performed by taking 100 µl from the third well to the fourth well and this procedure was repeated with the other 5 wells until reaching the desired concentration, i.e. (6.25 mg/ml). Aliquot of 100 µl of previously prepared bacterial broth were added to each well except the control one. All the plates were incubated at 37 ºC for 24 hr. The MIC was detected by the lack of turbidity in the wells. For the confirmation of growth inhibition, re-culture plates were incubated at 37 ºC for 24 hr.

2.7. Statistical analysis

Data analysis was performed using statistical software program (Graphpad Prism for Windows Version 5.0, GraphPad Software, Inc., Sandiego, CA, USA). Data were assessed for normal distribution using D'Agostino and Pearson omnibus normality test. Data were normally distributed; consequently mean and standard deviation for each assessed variable was calculated. Two-way ANOVA with Duncan *post hoc* multiple comparison tests was used to identify which group was statistically different from the rest. Differences between means at $p < 0.05$ were considered significant.

3. Results

The efficacy of ethanolic plant extracts varied significantly on the growth of tested bacteria (Table 1) ($p<0.05$). *Zizyphus Vulgaris, Cistus salvifolius, Thymus capitatus,* and *Arbutus pavarii* were the most effective, as the values of inhibition zones were 20 ±2.5 mm, 18 ±2.1 mm, 14 ±2.2 mm and 15 ±2.0 mm for *Staphyloccocus aureus* and 10 ±2.1 mm, 14.0 ±1.9 mm, 15 ±2.0 mm, and 15 ±1.7 mm for *Bacillus subtilis,* respectively. *Pistacia atlantica* and *Laurus nobilis* showed a poor effect against *Staphyloccocus aureus* and *Pistacia atlantica* and *Rhus tripartata* showed a poor effect against *Bacillus subtilis.*

Table 1: The inhibitory effect (mm) of ethanolic extracts of selected medicinal plants on the growth of *Staphyloccocus aureus* and *Bacillus subtilis*

Plant extract	*Staphylococcus aureus*	*Bacillus subtilis*
Zizyphus Vulgaris	20 ±2.5^{a*}	10 ±2.1^{a}
Laurus nobilis	10 ±1.3^{b*}	14 ±1.5^{b}
Thymus capitatus	14 ±2.2^{c}	15 ±2.0^{b}
Cistus salvifolius	18 ±2.1^{a*}	14.0 ±1.9^{b}
Arbutus pavarii	15 ±2.0^{c}	15 ±1.7^{b}
Rhus tripartata	16 ±1.8^{c*}	7 ±1.2^{c}
Pistacia atlantica	11 ±1.6^{b*}	9 ±1.4^{c}

^{a,b,c}: Means with different superscript letters in the same column are significantly different at p< 0.05. Each column represents the comparison between different plant extracts on each bacteria.

*: Means with different superscript letters in the same raw are significantly different at p< 0.05. Each raw represent comparative effect of each plant extract on both bacteria.

Cistus salvifolius extract caused an inhibition of *Staphyloccocus aureus* growth at 25 mg/ml, *Thymus capitatus, Arbutus pavarii, Rhus tripartata* and *Pistacia atlantica* at 50 mg/ml. However, the minimal inhibitory concentration was 200 mg/ml for other plant extracts (Table 2).

Table 2: Minimum inhibitory concentration exhibited by ethanolic extract of tested plants on the growth of *Staphyloccocus aureus*

Tested plant	Tested concentrations (mg/ml)					
	6.25	12.5	25	50	100	200
Zizyphus Vulgaris	+	+	+	+	+	-
Laurus nobilis	+	+	+	+	+	-
Thymus capitatus	+	+	+	-	-	-
Cistus salvifolius	+	+	-	-	-	-
Arbutus pavarii	+	+	+	-	-	-
Rhus tripartata	+	+	+	-	-	-
Pistacia atlantica	+	+	+	-	-	-

The minimal inhibitory concentration for *Bacillus subtilis* was 50 mg/ml for *Thymus capitatus, Arbutus pavarii* and *Pistacia atlantica* extract, 100 mg/ml for *Cistus salvifolius* and 200 mg/ml for other tested plant extracts (Table 3).

Table 3: Minimum inhibitory concentration exhibited by ethanolic extract of tested plants on the growth of *Bacillus subtilis*

Tested plant	Tested concentrations (mg/ml)					
	6.25	12.5	25	50	100	200
Zizyphus Vulgaris	+	+	+	+	+	-
Laurus nobilis	+	+	+	+	+	-
Thymus capitatus	+	+	+	-	-	-
Cistus salvifolius	+	+	+	+	-	-
Arbutus pavarii	+	+	+	-	-	-
Rhus tripartata	+	+	+	+	+	-
Pistacia atlantica	+	+	+	-	-	-

Petroleum ether extract of *Zizyphus Vulgaris* plant was the most effective, causing an inhibition zone of 14 mm on *Staphylococcus aureus* growth whereas other medicinal plant extracts showed no activity. However, Petroleum ether extract of *Cistus salvifolius* plant was the most effective antimicrobial agents against *B. subtilis* growth (9 mm) while other extracts showed no activity. *Zizyphus Vulgaris* extract was effective at 100 mg/ml and *Cistus salvifolius* was effective at 200 mg/ml.
Chloroform extracts of *Rhus tripartata* and *Thymus capitatus* were effective against *Staphylococcus aureus* at 100 and 200 mg/ml, respectively.

4. Discussion

Over the last years, a number of publications have been reported on the antibacterial activity of extracts from medicinal plants [20, 21]. However, in Libya, the antibacterial effect of different extracts of local medicinal plants has not been documented. Thus, in the current work, we investigated the antibacterial activity and determined the MIC of extracts of seven medicinal plant leaves on *Staphylococcus aureus* and *Bacillus subtilis.* The present results revealed that the ethanolic extract of selected plants was more effective compared to chloroform or petroleum ether extracts. There was a significant variation of the effect of different ethanolic plant extracts on the inhibition of selected bacteria. *Zizyphus Vulgaris* and *Cistus salvifolius* were the most effective against *Staphylococcus aureus*, where their ethanolic extracts produced an inhibition zone of 20 ±2.5 mm and 18 ±2.1 mm respectively with no bacterial growth was detected at 50 mg/ml for *Cistus salvifolius* and 200 mg/ml for *Zizyphus Vulgaris.*
The antibacterial activity of the plant may be attributed to the various phytochemical constituents present in the crude extract. Similar results was reported on *Cistus ladanifer* which has been found effective against five Gram-negative and Gram-positive bacteria including antibiotic resistant *staphylococcus spp.* [8]. However, *Cistus salvifolius* was found to have antioxidant effect [22]. Water extract of *Cistus salvifolius* has been found to have potent inhibitory effect against COX-1 and COX-2 which are mediators of inflammation [23].
Ethanolic extract of *Ziziphus vulgaris* was found more effective against *Staphylococcus aureus.* However, it has been found also effective against Gram-negative bacteria and has antioxidant effect [24], suggesting that it may has a broad spectrum antibacterial activity.
Laurus nobilis was found significantly more effective against *Bacillus subtilis* than *Staphyloccocus aureus.* Similar results was previously reported [25]. On the other hand, *Laurus nobilis* has not been found effective against bacteria [9].
Thymus capitatus provided approximately an inhibitory effect on the growth of *both Staphyloccocus aureus* and *Bacillus subtilis.* **S**imilar result was previously reported on ethanolic extract of this plant leaves [26]. Other studies indicated that *Thymus capitatus* has antifungal and antioxidant effects [27], as well as anthelmintic effect [28].
Similar to *Thymus capitatus, Arbutus pavarii* produced a significant equal inhibitory effect on both bacteria under investigation. Studies on other plant species *Arbutus unedo* extracts indicated that it has antimicrobial [8], and antioxidant effect [29].
Rhus tripartata extract provided a significant higher inhibitory effect on growth of *Staphyloccocus aureus* than *Bacillus subtilis* ($p<0.05$). *Rhus* root and bark extract could protect against ulcer due to its antioxidant and antisecretory effect [14]. In contrast, it was reported that ingestion of *Rhus spp.* could produce acute toxic encephalopathy [30].
Similar to *Rhus tripartata, Pistacia atlantica* was found effective against both bacterial isolates. In a study carried out on dental plaque bacteria and subgingival microorganisms, *Pistacia atlantica* could decrease significantly the aerobic bacteria [31]. Moreover, *Pistacia atlantica subspp. kurdica* was included as an antioxidant [32].
Alcoholic extracts of tested plants provided better antibacterial effect than other solvents. The effectiveness of the extracts largely depends on the type of solvent used as it has been reported that the organic extracts provided more powerful antimicrobial activity as compared to aqueous extracts [33]. It has also been reported that most of the antibiotic compounds already identified in plants are aromatic or saturated organic molecules which can easily solubilized in organic solvents [34].
Similar results showing that the best antimicrobial activity was obtained by the alcoholic extracts of *Leucas aspera* and *Holarrhena antidysenterica* [35], *M. azerarach* [33] and *Callistemon citrinus* and *Albizia lebbeck* [36]

The limitations of present study are that the use of two Gram-positive bacterial species only, the tested bacteria was not examined for antibiotic resistance based on molecular bases and the use of plant leaves only. Therefore, all these shortcomings should be considered in further studies.

5. Conclusion

The results of the present study indicate that ethanolic extract of *Cistus salvifolius, Arbutus pavarii, Thymus capitatus* and *Zizyphus Vulgaris* have potent effect on both *Staphyloccocus aureus* and *Bacillus subtilis. Cistus salvifolius* has been found highly effective against *Staphyloccocus aureus* with a MIC of 25 mg/ml. This study indicated that these plants could be a potential source of effective antibacterial agents. Further investigations need to be done on a wide range of bacteria and fungi to assess the spectrum of such plant extracts. Moreover, other parts of the examined plants are also need to be assessed for their antibacterial activity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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