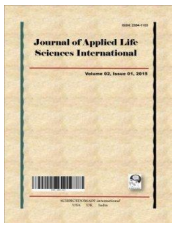

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Anti-bacterial Activity of Folk Medicinal Plant Extracts of Saudi Arabia on Isolated Bacteria

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Short Communication

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ABSTRACT

The antibacterial activity of four wild plants (*Ziziphus spina-christi*, *Citrullus colocynthis*, *Salvadora persica* and *Calligonum comosum*) used in folk medicine in Saudi Arabia was tested. The antibacterial activity of their ethanolic extracts were determined using the agar well diffusion technique. Two microorganisms were used, Gram-positive bacterium (*Bacillus subtilis*) and Gram-negative bacterium (*Escherichia coli*). Distilled water was used as the negative control. The results indicated that the ethanolic extracts of all four plants exhibited antibacterial activity. The aqueous extract was not effective when compared to the ethanolic extract. The inhibition zone was higher against the Gram-negative bacteria than the Gram-positive bacteria. The antibacterial activity of the leaf extract of *Calligonum comosum* demonstrated the highest activity (15 – 17 mm) compared to the other plant extracts. Minimum inhibitory concentration (MIC) values were also evaluated in this study. The MIC values obtained using the agar-dilution test ranged from 3.15 to 8.78 mg ml^{-1}. Further research aimed at elucidating the chemical constituents of these species will likely open new avenues, including the development of drugs.

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Keywords: Anti-bacterial activities; plant extracts; Bacillus subtilis; Escherichia coli.

1. INTODUCTION

Plants are frequently used in folk medicine since ancient times. Screening of these medicinal plants for antimicrobial activity is essential before extraction of various the bioactive compounds they possess. Plants produce a huge variety of important bioactive compounds such as phenols, quinones, flavones, tannins, coumarins, terpenoids, alkaloids for natural protection against microorganisms, insects, diseases and herbivores [1-5]. Plant-derived medicines are cheaper, more effective with least side effects [6]. Moreover, indiscriminate use of antibiotics has led to the emergence of multidrug resistant bacteria [7,8].

Calligonum comosum locally known as (Alarta) belongs to the botanical family *Polygonaceae* (Fig. 1). It is a plant of tropical and subtropical regions and is wide spread in Saudi Arabia and Arabian Peninsula**.** Several studies have confirmed the anti-bacterial activity and benefits of *C. comosum* leaves in the treatment of certain skin diseases [9,10]. *Salvadora persica* is an important medicinal plant, widespread in the world and Arabian Peninsula locally known as (Arak); it belongs to the botanical family *Salvadoraceae* (Fig. 2). Several studies have been done on the efficiency of aqueous and alcoholic extracts of *S. persica* against bacterial and fungal infections of the mouth [11,12,13,14].

Fig. 1. *Calligonum comosum* (Alarta)

Citrullus colocynthis is widely distributed in the desert and is locally known as (Hanzl) (Fig. 3). *C. colocynthis* belongs to the botanical family *Cucurbitaceae*. The fruit of *C. colocynthis* has been studied for its biological activities, such as antioxidant, cytotoxic, antidiabetic, antilipidemic, insecticide, antimicrobial and anti-inflammatory [15]. In addition, *C. colocynthis* seeds also have nutritional value in terms of protein and mineral content in addition the seeds are used to extract oils.

Fig. 2. *Salvadora persica* (Arak)

Fig. 3*. Citrullus colocynthis* (Hanzl)

Zizyphus spina-christi is a wild tree used as folk medicine. *Z. spina-christi* is frequently available in Saudi Arabia and is locally known as (Sidr) (Fig. 4). *Z. spina-christi* belongs to the botanical family *Rhamnaceae*. *Z. spina-christi* has been used in traditional medicine of many diseases in developing countries [16,17]. Moreover, the decoction extracted from the bark and fruits are used in wound healing and body wash. *Z. spina-christi* leaves are rich in alkaloids, saponin glycosides and several flavonoids [18,19].

The objective of this study was to examine the antibacterial activity of ethanol and aqueous extracts of *Z. spina-christi*, *C. colocynthis*, *S. persica*, and *C. comosum* against two phytopathogenic bacteria *Bacillus subtilis* and *Escherichia coli.*

Fig 4. *Ziziphus spina-christi*

2. MATERIALS AND METHODS

2.1 Collection of Plant Material and Preparation of Crude Extracts

Fresh plant material used in this study were: *Z. spina-christi* (leaves), *C. colocynthis* (fruits), *S. persica* (roots), and *C. comosum* (Leaves) collected from the north-east of Riyadh city, in "Dahna" area in Saudi Arabia. The fresh leaves and roots were cut into small pieces, washed in distilled water and air-dried in 75% shade at room temperature. After drying, the plant materials were ground to powder and extracted with hot water and ethanol: 10 g of the powdered sample was suspended in 100 ml of distilled hot water and 90% ethanol. The samples were then shaken and allowed to settle at room temperature for 72 and 42 hrs in hot water and ethanol, respectively, with manual agitation after every 24 hrs. Each extract was then filtered through Whatman filter paper. Each of the resulting filtrates was then concentrated by evaporation using a rotary evaporator under vacuum. The residue of each extract was kept in the refrigerator until used.

2.2 Source of Pathogens and Determination of Antibacterial Activity

B. subtilis and *E. coli* obtained from specialist hospital (King Khalid University Hospital, Riyadh, Saudi Arabia) were used for determination of antibcterial activity. This activity of the aqueous and ethanol extracts of the four plant sample was evaluated using the agar well dilution method [20]. The bacterial isolates were reconstituted and inoculated on nutrient agar plates. A sterile cork borer was used to bore 3 wells of 6 mm diameter on the nutrient agar plates. A 0.5 ml sample of each extract was introduced into the wells using a sterile pipette. Distilled water was placed in one of these wells as the negative control. The plates were then incubated at 37ºC for 18 to 24 hrs. Antibacterial activity was determined by measuring the diameter of inhibition zones. Each experiment was carried in four replicates.

2.3 Bacterial Susceptibility and Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory concentration (MIC) was determined by the micro-dilution method according to the National Committee for Clinical Laboratory Standards [21]. The extract was incorporated into the nutrient agar at concentrations ranging from 2.5 mg ml^{-1} to 20 mg ml^{-1}. A control without the extract was also set up. About 10 µl each of the test organisms, previously diluted to give 10^{6}cfu $ml^{-1}$was used to inoculate the plates. These were incubated at 37ºC for 24 hrs in the first instance, and for another 24 hours, before recording the growth observation. The MIC values (the lowest concentration of the extract which gave no bacterial growth) were determined after 28 hours.

3. RESULTS AND DISCUSSION

The profile of the four medicinal plants used in this study is shown in Table 1. The effect of the different plant extracts on the two test organisms is shown in Table 2. All the ethanolic extracts of *Z. spina-christi*, *C. colocynthis*, *S. persica* and *C. comosum* were inhibitory to the two test organisms used in this study (*B. subtilis* and *E. coli*). Table 2 shows the individual diameter (mm) zones of inhibition produced by the extracts. The results showed that the extracts are effective against bothtest organisms; the highest activity was demonstrated by ethanolic extract of *C. comosum* against *E. coli* (with 17 mm in diameter).

The lowest activity was recorded by the ethanolic extract of *S. persica* against *E. coli* (11 mm). The largest inhibition zone was found using the ethanol extract against *E. coli* (14 - 17 mm)

compared with *B. subtilis.* Results showed that, for all extracts tested, MIC values ranged from 3.15 mg ml^{-1} to 8.78 mg ml^{-1} (Table 3).

The results of the experiment showed significant antibacterial action of the ethanolic extracts against the tested microbes. The results indicated that the antibacterial activity of the leaf extract of *C. comosum* demonstrated the highest activity ranged from 15 to 17 mm against the test organisms compared to the other plant extracts. The obtained results were in agreement with [9] who reported that *C. comosum* had a significant antibacterial activity on *Listeria ivanovii* with growth inhibition zone ranging from 9 to 18 mm by the agar-well diffusion method. Moreover, [22,23] reported that the plants belonging to *Polygonaceae* family, such as *C. comosum,* possess antimicrobial properties directed against various pathogenic and nonpathogenic bacteria such as those belonging to *Staphylococcus, Pseudomonas, Escherichia, Bacillus, Klebsiella, Photobacterium, and Streptococcus* genera. The results also showed that the organic extract (ethanol) had significantly higher antibacterial activity compared with the aqueous extract (Table 2). It was reported that different solvents have different extraction capacities for phytoconstiuents [24]. On the other hand, significantly wider zones were observed between the Gram positive bacterium *B. subtilis* and the Gram negative bacterium *E. coli* for *C. colocynthis* and *S. persica* (Table 2). The inhibition zone was higher against Gram negative bacteria compared to Gram positive bacteria probably due to Gram negative bacteria being more sensitive than Gram positive bacteria, due to differences in the structure of the cell wall, such as its thickness [25,26]. In addition, the results revealed that the *Z. spina-christi*, *C. colocynthis*, *S. persica* and *C. comosum* extracts were effective against the test organisms, in agreement with the results already reported [27]. The MIC values differ between plant tissues and were dependent on the organic solvent used in the extraction [28]. In this experiment, *C. colocynthis*, *S. persica* ethanolic extrects had lower MICs than *Z. spina-christi* and *C. comosum*, for both bacterial organisms (Table 3).

Table 1. Used part of the selected plants

Botanical name	Family	Local name	Part used	Extract dry weight (g ml^{-1})
Ziziphus spina-christi	Rhamnaceae	Sidr	Leaves	10
Citrullus colocynthis	Cucurbitacea	Hanzl	Fruits	10
Salvadora persica	Salvadoraceae	Arak	Roots	10
Calligonum comosum	Polygonaceae	Alarta	Leaves	10

Table 2. Antibacterial activity of ethanolic and water extracts (inhibition zone, mm) against *Escherichia coli* and *Bacillus subtilis*

Plant name	Bacterial species			
	Escherichia coli		*Bacillus subtilis*	
	Ethanol	Water extract	Ethanol	Water extract
Ziziphus spina-christi	14±1.03 a	-	12±0.98 a	-
Citrullus colocynthis	16±0.88 c	0.5	14±0.64 a	0.2
Salvadora persica	16±0.68 c	0.4	11±1.87 b	-
Calligonum comosum	17±0.62 b	0.2	15±1.08 b	0.3

Values are mean ±SD of four replicates, values within a row with different alphabets are significantly different. (p≤0.05), (-) = not detected

Table 3. Minimal inhibitory concentration (MIC) in mg ml^{-1} of ethanol extract of plants against tested organism

Plant name	Bacterial species	
	Escherichia coli	*Bacillus subtilis*
Ziziphus spina-christi	8.78	6.25
Citrullus colocynthis	3.24	3.15
Salvadora persica	3.56	4.67
Calligonum comosum	6.25	6.78

Values are mean of four replicates

4. CONCLUSION

The results of this study indicated that the ethanol extracts were more effective for all four plants. And the extracts were active against Gram negative and Gram positive bacterium, for all four plant species tested: *Z. spina-Christi*, *C. colocynthis*, *S. persica*, and *C. comosum.* Further work needs to be done with more bacterial and fungal species, using more replicates, and compared with known antibiotics to validate these results. This study helpsto verify the antibacterial medicinal properties of these four plants in herbal medicine by rural people. Identification of the active compound in these plants are also required to assess their safety for human health as the plants are available and cheaper.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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