International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Antibacterial Activity of Three Medical Plant Extracts of Saudi Arabia on Isolated Bacteria

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Abstract

This study focuses the significant antibacterial activity of three medical plants (*Salvadora persica, Alluim sativum* and *Tamarix aphylla*) used in folk medicine in Saudi Arabia. The antibacterial activity of their ethanolic extracts were determined using the agar well diffusion technique. Two microorganisms were used, Gram positive *Staphylococcus areus* and Gram negative *Escherichia coli*, distilled water was used as the negative control. The result indicated that the ethanolic extracts of all tree plants exhibited antibacterial activity.

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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 *Salvadora persica* demonstrated the highest activity (14 – 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 3.45 to 6.25 $mg.ml^{-1}$. Further research aimed at elucidating the chemical constituents of these species will likely open new avenues, including the development of drugs.

Keywords: Anti-bacterial activities; plant extracts; *Staphylococcus areus; Escherichia coli.*

1. Introduction

Recently, a number of human pathogenic bacteria develop resistance to commonly used antibiotics, due to the indiscriminate use of antibiotics [1]. Furthermore, many antibiotics with different undesirable side effects have forced many scientists to look for new antimicrobial substances from various sources, e.g medicinal plants [2]. More so, according to world health Organization, more than 80%, of the world's population use traditional medicine for their primary healthcare needs [3]. Plants used in traditional medicine contain a wide range of substances. 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 [4;5;6;7].

Garlic (*Allium sativum* Linn.) has an important dietary and medicinal role for centuries. Its therapeutic uses include beneficial effects on the cardiovascular system, antibiotic, anticancer, anti-inflammatory, hypoglycemic, and hormone-like effects [8] Garlic extracts have been used to treat infections for thousands of years [9]. Its typical pungent odor and antibacterial activity depend on allicin, which is produced by enzymatic (alliin lyase) hydrolysis of alliin after cutting and crushing of the cloves [10]. Antibacterial activity and reducing activies of garlic extracts is attributed to the presence of Thiosulphide and allymethyl sulphide. Their disruption cell components and their ability to block enzyme pathways have implicated in bacterial inhibition [11,12].

Salvadora persica L., commonly named miswak, belong to the family known as Salvadoraceae. It has been used by many Islamic communities as chewing sticks, and has been scientifically proven as being very useful in the prevention of tooth decay, even when used without any other tooth cleaning methods [13] Chewing sticks gotten from the roots, twigs, or stems of S. *persica* are commonly used in the Middle East, as a means of maintaining oral hygiene. Studies show that *S.persica* extracts can be somewhat compared to other oral disinfectants, and anti-plaque agents, such as triclosan, and chlorhexidine gluconate, if usedat a very high concentration [14;15]. It has been reported that extracts from miswak, possess various biological properties, containing significant antifungal [16], and antibacterial effects, especially against bacteria considered important for development of dental plaque [17].

Tamaricaceae (the Tamarix family) showed antimicrobial activities against a range of pathogenic bacteria and have many chemical compounds; for example, 62 different chemical compounds were identified in *Tamarix boveana*, and this plant has antimicrobial activity against six Gram-positive bacteria and four fungi [18].

Taramix aphylla has traditionally been used in folk medicine to cure various ailments including hepatitis, aczema and skin diseases such a *Tinea capititis* and *syphilis* [19]. Alcoholic extracts of *T. aphylla* have been shown to be antioxidant; acting *T. aphylla* methanolic extract been shown to have protective effect in terms of human health [20]. If the smoke from burnt leaves of *T. aphylla* passed overwound, it may cause healing and the leaves in powdered from have traditionally been used to treat toothache [21]. [22] elucidated six new structures of the tannis in galls of *T. aphylla* (L.). Previously, composition of polyphenols (phenolic acids and flavonoids) of *T. aphylla* which is a sources of bioactive compounds has been studied, and it was found that leaves contain higher amount of polyphenols than the stems [23]. The current study was conducted to examine the antimicrobial activity of several concentrations of *Salvadora indica, Alluim sativum* and *Tamarix aphylla* extract against two pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli.*

2. Materials and Methods

2.1. Collection of plant material

Fresh plant samples used in this study were: *Tamarix aphylla* leaves, *Salvadora persica* roots and of *Allium salivum* bulbs.

Taramix aphylla leaves, *Salvadora persica* roots were collected from the Riyadh habitat, Kingdom of Saudi Arabia. Fresh garlic (*Allium salivum)* bulbs were purchased from local market (Table 1)

Table 1: Information on the plants and parts used

Species (Family)	Local name	Part used	**Extract dry weight(g.ml^{-1})**
Salvadora persica	Arak	Roots	10
Alluim sativum	Thoum	Bulbs	10
Tamarix aphylla	Athal	Leaves	10

The garlic bulbs were peeled and cleaned. Cleaned cloves were surface-sterilized by immersing them into 90% (v/v) ethanol for 60s [24] Residual ethanol on surface was evaporated in sterile laminar airflow chamber followed by homogenizing aseptically in sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth. This extract was considered as the 100% concentration of the extract.

The *Taramix aphylla* fresh leaves and *Salvadora persica* roots were cleaned and washed with distilled water; air dried and were chopped and ground into powder and passed through sieved of 0.5mm mesh screen and kept separately in clean polyethylene bags. The powder were than extracted with hot water and ethanol: 10 grammes 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 hours in hot water and ethanol, respectively, with manual agitation after every 24 hours. 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 at 4^{o}C for further use in terms of the antimicrobial activity.

2.2. Pathogenic strains

Two bacterial strains including *E. Coli* and *Staphylococcus aureus* were obtained from the Microbial Laboratory, Faculty of Sciences, Princess Norah University, Kingdom of Saudi Arabia. The strains were used to determine the antibacterial activity. This activity was evaluated using the agar well dilution method.

2.3. Testing of antibacterial activity using agar well diffusion method

The bacterial isolates were reconstituted and inoculated on nutrient agar plates. A sterile cork borer was used to bored 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 37^{o} C for 18 to 24 hours. Antibacterial activity was determined by measuring the diameter of inhibition zones. Each experiment was carried in four replicates.

2.4. 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. 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 10ul each of the test organisms, previously diluted was used to inoculate the plates. These were incubated at 37C for 24 hours. in the first instance, and for another 24 hours, before recoding the growth observation. The MIC values (the lowest concentration of the extract which gave no bacterial growth) were determined after 28 hours.

2.5. Statistical analysis

Data management and analysis were made using SPSS version 16.0 for windows. P values <0.05 were considered as statistically significant.

3. Results

Concentration of the three plant extract tested against *E.Coli* and *Staphylococcus aureus* for their antibacterial activities and the result are given in table 2.

The ethanolic plant extract were inhibitory to the two test organisms used in this study. Table 2 shows the individual diameter (mm) zones of inhibition produced by the extracts. The result showed that the extract are effects against both organism; the highest activity was demonstrated by ethanolic extract of garlic against E.Coli (with 18mm in diameter).

The lowest activity was recorded by the ethanolic extract of Tamarix against Staphylococcus (11mm). The largest inhibition was found using the ethanolic extract against E.coli (12-17mm) compared with *Staphyloccus areus* (11-14mm).

Table 2: Antibacterial activity of ethanolic and water (inhibition zone, mm) against *Escherichia coli* and *Stapylococcus aureus*

Species	**Bacterial species**			
	Esherchia coli		*Staphylococcus aureus*	
	Ethanol	Water	Ethanol	Water extract
Salvadora persica	17±0.51	0.3	14±0.64	-
Alluim sativum	18±0.36	0.5	14±0.89	0.2
Tamarix aphylla	12±02	0.5	11±302	0.2

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

The result of the experiment showed significant antibacterial action of the ethanolic extracts against the tested microbes.

A study evaluated the antibacterial effects of *Salvadora persica* effects against two bacterial types *E.coli* and *S. aureus*. The obtained *Salvadora* root extract exhibited considerable inhibitory effects against the two tested bacteria with inhibitory zones varying between 14 and 17mm. Several studies [17] have been either done on the efficiency of aqueous and alcoholic extracts of *S. persica* against bacterial and fungal infections.

The result indicated that the antibacterial activity of the leaf extracts of *garlic* demonstrated the highest activity ranged from 14 to 18 mm against the test organisms compared to the other plant extracts. The obtained results were in agreement with [25] who reported that *garlic* had a significant antibacterial activity on *Staphylococcus* with growth inhibition zone ranging from 9 to 18 mm by the agar-well diffusion method. Moreover, [11] reported that the garlic possess antimicrobial properties directed against nine anaerobic microaerophilic and aerobic bacteria (*Bacteroides fragillis, Clostridium perfringens, Enterobacter cloacae, Escherichia coli, Pseudomonas aeuginosa, Salmonella typhimurium* and *Staphyloccus aureus).* The result also showed that the organic extract (ethanol) had significantly higher antibacterial (Table 2). It was reported that different solvents have different extraction capacities for phytoconstiuents [6]. On the other hand, significantly wider zones were observed between the Gram positive bacterium *S. aureus* and the Gram negative bacterium *E. coli* for *garlic* 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 [27]. In additional, the result revealed that *Alluim sativum, S. persica* and *tamarix* extracts were effective against the test organisms, in agreement with the result already reported [28; 17].

Result showed that, for all extracts tested, MIC values are ranged from 3.45 $mg.ml^{-1}$ to 6.25$mg.ml^{-1}$. The MIC values differ between plant tissues and were dependent on the organic solvent used in the extraction [26]. In

this experiment, *Tamarix aphylla* ethanolic extracts had lower MICs than *Alluim sativum* for both bacterial organisms (Table 3).

Table 3: Minimal inhibitory concentration (MIC) in $mg.ml^{-1}$ of ethanol extract of plants againstTested Organism

Species	Bacterial species	
	Escherichia coli	*Staphylococcus aureus*
Salvadora persica	4.52	4.01
Alluim sativum	6.25	6.02
Tamarix aphylla	3.65	3.45

Values are means of four replicates

4. Conclusion

The result 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 three plant species tested: *Allium, Tamarix and Salvadora persica.* 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 helps to 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.

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