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Phytochemical screening and antibacterial activity of crude aqueous and ethanol extracts of *Salvadora persica* L. stem (Miswak) from Saudi Arabia

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ABSTRACT

Since long time ago, medicinal plant products have been a rich source of antibacterial drugs. *Salvadora persica* L. stem is used basically for cleanse, get rid of microbes and the stink smell of the mouth. Phytochemical investigations on the aqueous and ethanol extracts of *Salvadora persica* L. stem revealed presence of some bioactive principles, such as Saponins, alkaloids, cardiac glycosides, Terpenoids and flavonoids. Antimicrobial investigation revealed presence of some degree of antimicrobial effect against some gram negative bacteria (*Salmonella enterica* ATCC 5174, *Proteus vulgaris* ATCC 49132, *Klebsiella pneumonia* ATCC 27736, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and some gram positive bacteria (*Bacillus cereus* ATCC 10876, *Staphylococcus epidermidis* ATCC 49461, *Staphylococcus aureus* ATCC 25923). This antibacterial activity considered Competitor to penicillin G, but modest when compared to gentamicin. The antibiotic penicillin is collapsing and it is no longer a good option.

Keywords: Phytochemical Screening, Antibacterial Activity, *Salvadora persica*, Miswak, Plant Extracts.

INTRODUCTION

Plant-derived antimicrobial agents could have advantages over the synthetic or semi-synthetic antibiotics by reducing the intensive use of antibiotics and reducing the development of antibiotic-resistant microorganisms [1]. The Chewing stick, Miswak or Siwak is mainly comes from *Salvadora persica* L. (family Salvadoraceae), a famous medicinal plant used for oral hygiene among Muslim Communities all over the world, it is cut from a small tree known in Arabian countries as Arak shrub. These chewing sticks could be made from stems, roots or twigs of *Salvadora persica* but the stem is major [2, 3]. This plant was used since ancient civilizations by Babylonians in Iraq, about 7000 years ago followed by Egyptians, Jews, Romans, and Islamic empires [3]. *Salvadora persica* (*S. persica*) has many therapeutic properties, it is used in traditional medicine systems as anti-plaque, anti-microbial, anti-inflammatory, anti-pyretic, analgesic, astringent and diuretic [4]. Other parts of this plant are also employed, fresh leaves are eaten as vegetables and used as anti-cough, anti-asthma and in treatment of rheumatism, its fragrant flowers are used as stimulant and purgative [5]. Many important minerals, chemical and phytochemical compounds were found in *S. persica* such as fluoride, calcium, phosphorous, pyrrolidine, glycosides, alkaloids, flavonoids, tannins, saponins and vitamin C [6].

The chewing sticks of Miswak has received much attention in Saudi Arabia, particularly among millions of Muslim pilgrims who come each year to the holly cities; Mecca and Medina in Saudi Arabia and buy a lot of these chewing sticks (Miswak). Accordingly, this study was aimed to investigate some phytochemical compounds of bioactive properties and screening for the antibacterial activity of the *Salvadorapersica* stem (*S. persica*), collected from Saudi Arabia.

MATERIALS AND METHODS

Plant material

Fresh samples of *S. persica* (stems with leaves) were collected manually from Jazan area, southern Saudi Arabia, by Mr. Khalid Assaf Al-Harbi and authenticated later by taxonomist; Dr. Gamal Al-Ghazali, at the Department of Laboratory Sciences, College of Sciences and Arts at Ar-Rass, Qassim University. Stems of *S. persica* were separated, cut into small parts and dried in shade for up to one month.

Plant extraction

Extraction was carried out using a maceration method as reported by Sami *et al.* [7], with some minor modifications. The dried stems of *S. persica, were* crushed into fine powders. 50 g of the stem's powder

was soaked in 500 ml of absolute Ethanol (Scharlau Chemie S.A.), another 50 g of this powder was soaked in 500 ml distilled water, kept in a dark well tighten glass bottles for up to 3 days at room temperature (35-37 °C) with frequent shaking. Then, the plant macerates were filtered using Whatman filter paper No.1 and evaporated to dryness under reduced pressure at 40 °C, the semi-solid residues were put in the incubator for up to 2 weeks for water extract and one month for ethanol extract at 45 °C until solvent totally evaporated, this is because ethanol as a solvent is lethal for bacteria and any ethanol residues in the extract may lead to deceptive results. Extracts were kept in refrigerator until used. For the antibacterial testing, the dry aqueous extract was reconstituted in 10% Dimethyl sulphoxide (Techno Pharmchem Haryana), while the dry ethanol extract was reconstituted in absolute methanol (Fisher Chemical).

Phytochemical analysis

Qualitative analysis of selected phytochemical tests was performed as described by Deshpande [8] for detection of alkaloids (Mayer's test), saponins (Foam test), tannins (Ferric chloride test) and cardiac glycosides (Killer Kiliani test). Flavonoids test was done as mentioned in Mujeeb *et al*. [9]. Terpenoids test was carried out as published in Veerachari and Bopaiah [10].

Referenced Microbial Strains

Eight referenced bacterial strains representing both Gram-negative and Gram-positive bacteria were used in this study, for evaluating the antibacterial activity of aqueous and ethanol extract of *S. persica* stems. The Gram negatives were *Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 5174, *Klebsiella pneumonia* ATCC 27736, *Pseudomonas aeruginosa* ATCC27853 and *Proteus vulgaris* ATCC 49132. The Gram-positives were *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC 49461 and *Bacillus cereus ATCC* 10876. Strains were purchased from Watin-Biolife, KSA.

Inoculum Preparation

Nutrient broth (Watin-Biolife, KSA) was used for growing the bacterial strains and diluting suspensions. Bacterial strains were cultured in nutrient broth for up to 18 h at 37 °C to reach the exponential phase and then adjusted to Mcfarland standards, equivalent to a final density of 2 x 10^8CFU/ml and served as working bacterial culture.

Determination of antibacterial activity

The antibacterial activity of ethanol and aqueous stem extracts of *S. persica* were determined by disc diffusion method as described by Doughari [11], with some modifications. In aseptic conditions, 100µl from each working bacterial cultures were mixed with a bottle containing 20 ml warm autoclaved Mueller-Hinton Agar (Watin-Biolife, KSA) was poured into 90 mm sterile disposable plates (Jalil Medicals) and left to solidify at room temperature. 6 mm discs previously prepared from Whatman No.1 filter paper and saturated with 200 mg/ml (about 4 mg/disc and the paper disc trap about 20 ul of the extract) of ethanol and aqueous extracts were loaded on the seeded Mueller-Hinton Agar plates. 6 mm antibiotic discs; Gentamicin 10µg and Penicillin G 10 units (Oxoid) were loaded on the plates and served as positive control. Seeded plates were incubated overnight at 37°C. The test repeated twice. Subsequently, the mean zone of inhibition was measured in millimeter (mm) using a ruler, 6 mm zone diameter considered as no inhibition.

RESULTS & DISCUSSION

The preliminary phytochemical screening of aqueous and Ethanol extracts of *Salvadora persica* stem was carried out in order to investigate the bioactive principles which may be the main factor behind the antibacterial activity. As presented in Table1, the aqueous extract showed the presence of saponins, alkaloids and cardiac glycosides, while the ethanol extract showed the presence of terpenoids and flavonoids. Plants produced numerous diverse bioactive compounds which vary between plant species. Saponins are glycosides widely exist in plants and it is believed to contain antibacterial compounds [12]. Alkaloids are a large, diverse group of secondary metabolites, reported as antimicrobial, it is able to intercalate with the microbial DNA [13]. Terpenoidsare reported to have anti-inflammatory, anti-malarial, anti-viral and antibacterial activity [14]. Flavonoids are hydroxylated phenolic compounds produced by many plants to combat bacterial infections by interacting with the bacteria cell wall and proteins [15]. Our results are in agreement –partially or totally-with previous studies on stem of *Salvadora persica* which showed that it has some or all of these bioactive phytochemical compounds: terpenoids, flavonoids, tannins, alkaloids and saponins [3, 4, 6, 16]. The antibacterial potential of aqueous and ethanol extract of stem of *Salvadora persica* was investigated. As shown in Table 2 and Figure 1, Aqueous extract exhibited some degree of antibacterial effects on gram negative bacteria which are *Pseudomonas aeruginosa* (10.5 ± 0.5 mm), Both of *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris* (9.5 ± 0.5 mm) and *Salmonella enterica* (8.5 ± 0.5 mm). As well, the ethanol extract showed some degree of antibacterial effects on gram negative bacteria which are *Salmonella enterica* (13.0 ± 1.0 mm), *Klebsiella pneumonia* (9.5 ± 0.5 mm), both of *Escherichia coli* and *Pseudomonas aeruginosa* (9.0 ± 0.0 mm), and *Proteus vulgaris* (8.0 ± 0.0 mm). These results when compared with Gentamicin are considered generally modest antibacterial activity. Good antibacterial activity should be above 10 mm zone of inhibition if used 6 mm disc [17]. Results presented in Table 3 and Figure 2 showed that aqueous extract of *Salvadora persica* stems recorded some degrees of antibacterial effects on gram positive bacteria which are *Bacillus cereus* (10.5 ± 1.5 mm), *Staphylococcus aureus* (9.5 ± 0.5 mm) and *Staphylococcus epidermidis* (6.5 ± 0.5 mm), respectively. Also, ethanol extract of *Salvadora persica* stems recorded some degrees of antibacterial effects on gram positive bacteria which are *Bacillus cereus* (9.0 ± 1.0 mm), *Staphylococcus aureus* (8.5 ± 0.5 mm) and *Staphylococcus epidermidis* (6.5 ± 0.5 mm), respectively. It's a modest result compared to Gentamicin. Interestingly, a closer look at Tables 2 and 3, and Figures 1 and 2, conclude that penicillin G is no longer effective antibiotics for both of gram negative and gram positive bacteria. Currently, the antibiotics are losing their effectiveness worldwide and necessitating surveillance program and research interest [18]. Our findings are in agreement with Abdelrahman et al. [19], who reported that *Salvadora persica* extracts exhibited low antimicrobial activity against tested bacteria when compared with 0.2% aqueous chlorhexidine. On the other side, our results disagree with AlLafi and Ababneh [20], who cited that *S. persica* recorded strong antimicrobial effects on the growth of *Streptococcus* sp. and *Staphylococcus aureus*. Differences in results may be related to the Geographical distribution of the pant, type of extractions and solvents used. Although, the antibacterial Efficiency of *S. persica* (Miswak) against different bacterial strains are well documented in literature [2, 16]. More advanced microbiological studies in vitro and *in vivo* are required, as there are study revealed that significant reduction in the bacterial count from the oral cavity was observed when using *Salvadora persica* (Miswak) mouthwash when compared with placebo one [21]. In General, the presence of the previously mentioned phytochemical compounds in the extracts may be responsible for the antibacterial activity of the chewing sticks of *Salvadora persica* against the tested bacterial strains. This also provides the scientific evidence for its traditional uses in cleansing oral cavity and teeth in the Muslim community in particular.

Table 1: Phytochemical screening of aqueous and ethanol extracts of *Salvadora persica* L. Stem

Plant extract	Saponins	Tannins	Alkaloids	Terpenoids	Flavonoids	Anthraquinones
Aqueous	+	–	+	–	–	–
Ethanol	–	–	–	–	+	–

Table 2: Antibacterial activity of different extracts against gram negative bacteria of Salvadorapersica L. stem compared to gentamicin and penicillin

Tested	Mean zone of inhibition (mm) of tested bacteria (Mean±SEM)*				
	SL	PR	KP	EC	PS
Aqueous 4 mg/disc	8.5 ± 0.5	9.5 ± 0.5	9.5 ± 0.5	9.5 ± 0.5	10.5 ± 0.5
Ethanol 4 mg/disc	13.0 ±1.0	8.0 ± 0.0	9.5 ± 0.5	9.0 ± 0.0	9.0 ± 0.0
Gentamicin 10µg/disc	12.0 ± 2.0	18.0 ± 0.0	21.0 ± 1.0	19.5 ± 0.5	20.5 ± 0.5
Penicillin G 10 units/disc	9.5 ± 0.5	–	8.5 ± 0.5	11.0 ± 1.0	7.5 ± 1.5

* Mean±standard error of means, mm= millimeter, – = No inhibitory activity or zone diameter 6mm. SL = Salmonella enterica ATCC 5174, PR = Proteus vulgaris ATCC 49132, KP = Klebsiella pneumonia ATCC 27736, EC = Escherichia coli ATCC 25922, PS = Pseudomonas aeruginosa ATCC 27853.

Table 3: Antibacterial activity of different extracts against gram positive bacteria of *Salvadorapersica* L. stem compared to gentamicin and penicillin

Extract	Mean zone of inhibition (mm) of tested bacteria (Mean±SEM)*		
	BC	SE	SA
Aqueous 4 mg/disc	10.5 ± 1.5	6.5 ± 0.5	9.5 ± 0.5
Ethanol 4 mg/disc	9.0 ± 1.0	6.5 ± 0.5	8.5 ± 0.5
Gentamicin 10µg/disc	14.5 ± 0.5	10.5 ± 0.5	13.5 ± 0.5
Penicillin G 10 units/disc	8.5 ± 0.5	–	8.5 ± 0.5

* Mean±standard error of means mm= millimeter, – = No inhibitory activity or zone diameter 6mm. Bc = Bacillus cereus ATCC 10876, SE = Staphylococcus epidermidis ATCC 49461, SA =Staphylococcus aureus ATCC 25923.

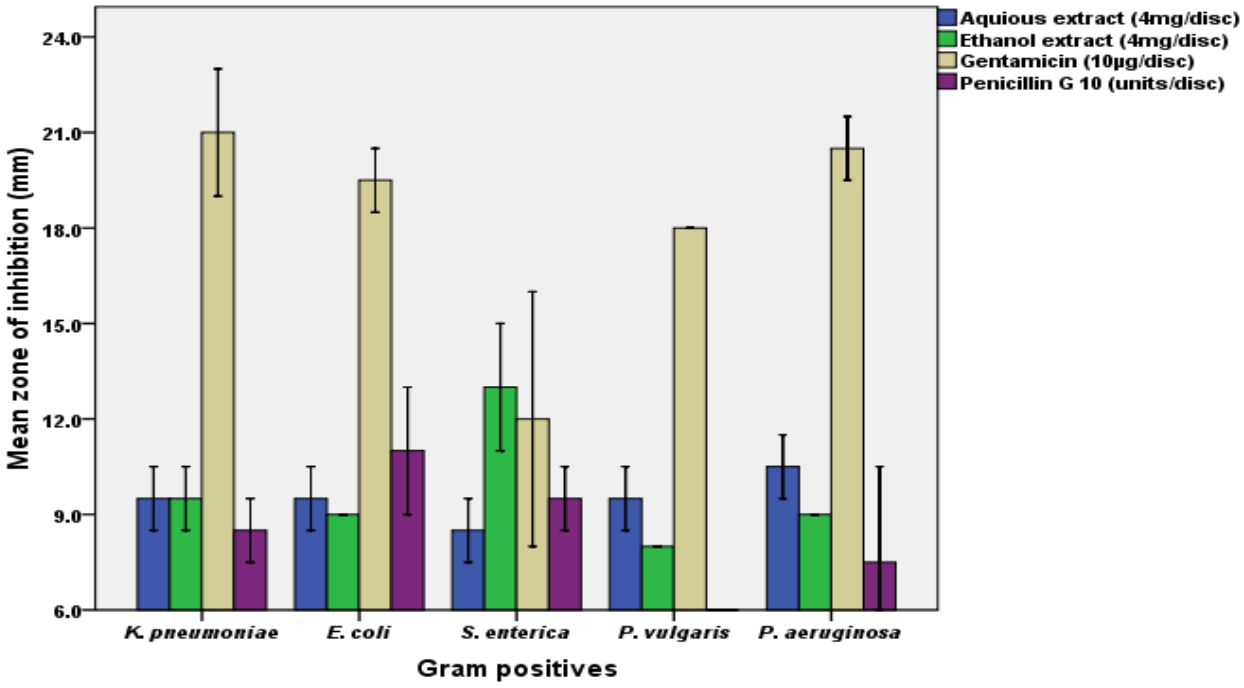

Figure 1: Antibacterial activity of different extracts against gram negative bacteria of *Salvadora persica* L. stem compared to gentamicin and penicillin

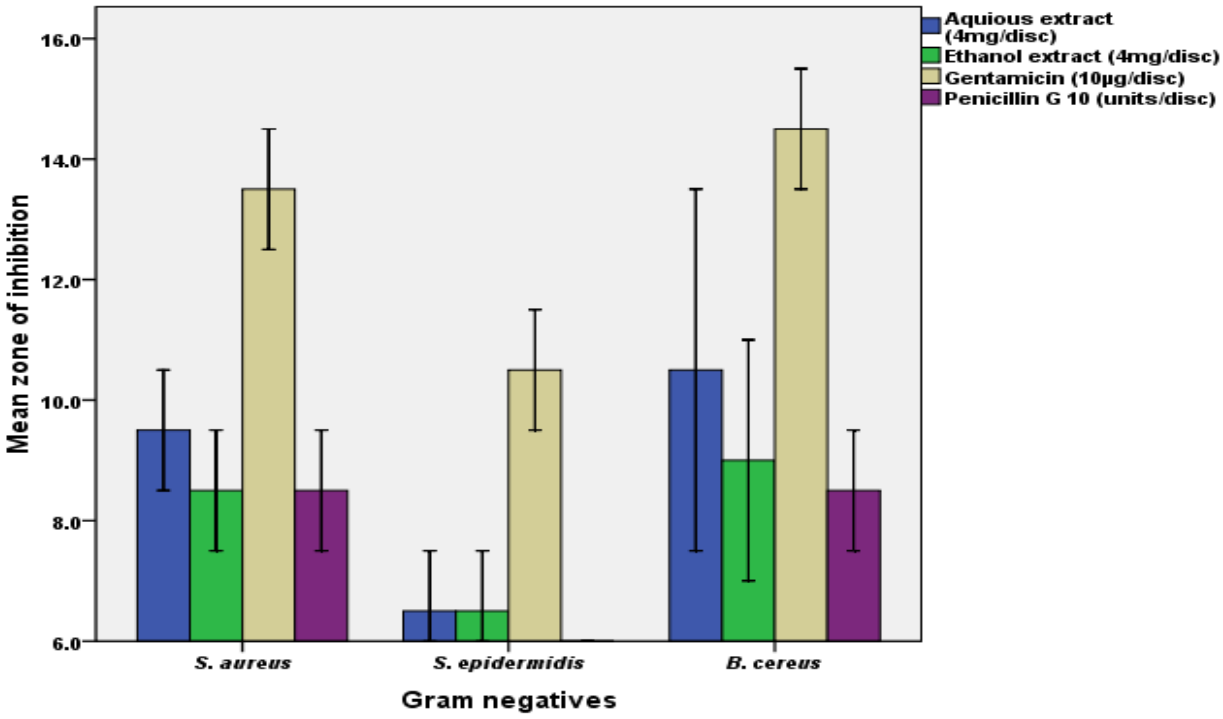

Figure 2: Antibacterial activity of different extracts against gram positive bacteria of *Salvadora persica* L. stem compared to gentamicin and penicillin

CONCLUSION

In conclusion, screening of medicinal plants is not only to find out the scientific rationale for their usage, but also to contribute in the global scientific efforts toward exploring new antibiotics and antimicrobial drugs to eradicate the growing phenomenon of multi-drug resistant microorganisms. Stems of *Salvadora persica* which are used traditionally in oral hygiene exhibited some degree antibacterial activity. Accordingly, it is recommended for considering promising candidate in Phyto-pharmacological industries such as toothpastes, mouthwashes and as a cleansing substance for oral cavity and teeth.

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