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Antibacterial Activity ofMiswak(SalvadorapersicaL.)Stem Aqueous and Ethanolic Extracts in vitro

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Abstract:

The aim was to evaluate the in vitro antibacterial activity of three concentrations (50, 75 and 100 µg/ml) of aqueous and ethanoliccrudeextractsofmiswak(Salvadorapersica) on threepathogenic bacteria (Staphylococcus aureus, Streptococcus pyogenesand Pseudomonas aeruginosa)using agar well-diffusion method.Phytochemical analysis revealed that the extracts contained alkaloids, flavonoids, phenols, tannins and steroids. Antibacterial evaluations demonstrated that both extracts inhibited the growth of the investigated bacteria in a concentration-dependent manner, but the aqueous extract was more efficient than the ethanolicextract.Thehighest growth-inhibition (GI) zone(22.1± 2.3mm)was observed in the culture ofP. aeruginosaat the concentration 100 µg/mlof the aqueous extract, while the lowest (12.3± 1.2 mm) was inthe culture of S.aureus using the ethanolic extract at the concentration 50 µg/ml. These findings suggest the antibacterial potential of Salvadorapersica stem, which might be related to its chemical constituents..

Keywords: *Salvadorapersica*, Chemical analysis, Antibacterial activity.

الخلاصة:

الهدف من هذه الدراسة تقييم الفعالية المضادة للبكتريا خارج الجسم الحي باستخدام ثلاثة تراكيز للمستخلصات المائية والكحولية لنبات المسواك (100,75,50 مايكروغرام/مل) ضد البكتريا المرضيـــة (Staphylococcus aureus, Streptococcus pyogenesand Pseudomonas aeruginosa) باستخدام طريقة الانتشار في الجل،بينت نتائج الكشف الكيميائي لهذه المستخلصات وجود المركبات الفعالة (القلويدات،الفلافونات،الفينولات،التانينات،الستيرويدات) واظهرت التقييمات المضادة للبكتريا امكانية كلا المستخلصين في تثبيط نمو البكتريا المدروسة اعتماداً على نوع التركيز وكانت فعالية المستخلص المائي اكبر من المستخلص الكحولي، ولوحظ اعلى تثبيط للنمو في منطقة (GI)في الوسط الزرعي لبكتريا ((22.1± 2.3mm) للتركيز 100مايكروغرام/مل للمستخلص المائي بينما كان الاوطأ (12.3± 1.2 mm) للوسط الزرعي لبكتريا (S.aureus) بأستخدام المستخلص الكحولي للتركيز 50 مايكروغرام/مل. من خلال هذه النتائج أُقترح امكانية فعالية ساق بنات المسواك كمضاد للبكتري اعتماداً على محتواه الكيميائي.

Introduction:

SalvadorapersicaL. of the family Salvadoraceaeis a small tree or shrub with a crooked trunk, seldom more than one foot in diameter. Its bark is scabrous and cracked, whitish with pendulous extremities. It has a pleasant fragrance, as well as a warm and pungent taste (1). Miswak (siwak and several other synonyms) is an Arabic word for the plant S.persicathatmeans tooth cleaning stick. In English, miswak has been mentioned as the "natural toothbrush(2),and its use can be traced back at least to pre- Islamic times (3). In Ayurvedic system of medicines, the plant is reported to have potent activity for dental complaints, and presently, many of the world populations including India and some Arab regions prepare tooth sticks from this plant (4). The stick is chewed or tapered at one end until it becomes frayed into a brush, and folkloric medicine suggest its cleaning and antiseptic potentials(5). Moreover, scientific investigations have suggested that miswakhas a number of medically beneficial properties including abrasives, antiseptics, astringent, detergents, enzyme inhibitors, and fluoride. Therefore, the

present study aimed to determine the general chemical constituents of the plant stem and to evaluate its antibacterial activity against pathogenic bacteria in vitro.

Materials and Method:

Plant collection and identification: The plant stems were purchased from a local market in Baghdad, and it was identified as SalvadorapersicaL. in the Herbarium of College of Agriculture (University of Baghdad).

Plant extraction: The dried plant stems were powdered using a coffee grinder, and then the powder was extracted with two types of solvents (distilled water or ethanol). In both cases, 50 grams of the processed plant were extracted in 250 ml of the solvent using the Soxhlet apparatus for six hours and the source of heating was a warm water bath (45°C). The obtained extract solution was then evaporated at 45°C using a rotary evaporator, and the resultant crude extract was frozen at -20°C until use to prepare the required concentrations (6). Three concentrations were tested for each extract (50, 75 and 100 µg/ml), which were prepared by dissolving the required material in sterilized distilled water.

Phytochemical analysis: Phytochemical analysis of all the two extracts was conducted following the procedure of Indian pharmacopoeia (7). These analyses included the detection of alkaloids, flavonoids, phenols, tannins and steroids in aqueous and ethanolic extracts.

Pathogenic bacteria: Three species of pathogenic bacteria (Staphylococcus aureus, Streptococcus pyogenesand Pseudomonas aeruginosa) were tested to determine the antibacterial activity of both extracts. The isolates of these bacteria were collected from the Microbiology Unit at Al-Yarmouk Teaching Hospital Laboratories.

Determination of Antibacterial Activity: The antibacterial activity of the two extracts at three concentrations (50, 75 and 100 µg/ml) was determined using the agar well diffusion method (8). Briefly, 5 mm diameterwells were punched in Nutrient agar with a cork borer and filled with 5 µl of the plant extract. Control wells containing distilled water (negative control) and standard antibiotic solution (Chloramphenicol, 100 µg/ml: positive control) were also included. The plates were incubated at 37°C for 24 hours, and thenthe antibacterial activity was assessed by measuring the diameter (mm) of growth-inhibition (GI) zone. All tests were replicated six times.

Statistical analysis: The GI zone was presented as mean ± standard deviation (SD), and differences between means were assessed by ANOVA (analysis of variance) followed by Duncan test that was set-up at $P \leq 0.05$ as a significant level. The SPSS (statistical package for social sciences) version 13.0 was employed to carry out such analysis.

Results:

Phytochemical analysis revealed that the aqueous and ethanolicextractswere positive for alkaloids, flavonoids, phenols, tannins and steroids, but a stronger reaction was observed for tannins in aqueous extract (Table 1).

Table 1: Phytochemical analysis ofmiswak (SalvadorapersicaL.) stem aqueous and ethanolicextracts.

Chemical Constituent	Reaction	
	Aqueous Extract	Etanolic Extract
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Tannins	++	+
Steroids	+	+

Both extracts exhibited a strong anti-bacterial activity against the three investigated pathogens as assessed by the diameter of GI zone. However, such activity was dependent on the type of extract, concentration and the bacteria under investigation.In general, larger GI zones were observed in cultures treated with the aqueous extract than in cultures treated with ethanolic extract, and the GI zone positively paralleled the diameter of zone. The most sensitive bacteria was P. aeruginosa, in which the GI zone diameter was 22.1±2.3 mm at the concentration 100 µg/ml of aqueous extract, while the lowest diameter (12.3±1.2 mm) was observed in the culture of S. aureus at the concentration 50µg/ml of aqueous extract, and the difference was significant ($P \leq 0.05$). The other diameters of GI zones were distributed between these two extremes (Table 2).

Table 2: Antibacterial activity of miswak (SalvadorapersicaL.) stem aqueous and ethanolic extracts.

Bacteria	Growth Inhibition Zone (mm: Mean ± SD)					
	Aqueous extract			Ethanolic extract		
	100 µg/ml	75 µg/ml	50 µg/ml	100 µg/ml	75 µg/ml	50 µg/ml
Staphylococcus aureus	18.3±5.6 Ba	15.4±3.4 Bb	14.1±3.6 Bb	15.2±2.5 Bb	13.5±3.1 Abc	12.3±1.2 Bc
Pseudomonas aeruginosa	22.1±2.3 Aa	20.9±37 Aa	17.5±2.5 Ab	18.2±3.5 Ab	17.4±2.9 Ab	15.1±1.8 Ac
Streptococcus pyogenes	18.7±3.6 Ba	17.5±5.2 Bab	16.4±3.1 Ab	16.5±2.7 Bb	15.2±2.3 Ab	15.6±12.9 Ab

Different upper case letters: Significant difference ($P \leq 0.05$) between means of columns.
Different lower case letters: Significant difference ($P \leq 0.05$) between means of rows.

Discussion:

In present investigation, the antibacterial activity of S. persicastemaqueous andethanolicextractswasassessedand a strong antibacterial activity was demonstrated. Both extractswere very active against the three tested bacterial strains, but their effectiveness varied, and the aqueous extract was more presented. This is in agreement with other studies, whichhave reported that S. persica extracts were effective against Streptococcusspp. even at lowconcentrations [9, 10]. This is reasonedby the fact that the aqueous extracts of S. persica contains important phytoconstituents such as vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides [11,12], which are known to possess significant antimicrobial activity [13,14]. Also, it has been reported that the aqueous extractof S. persicachewingsticksinhibited the growth of S.faecalis [15]. In a further study from Jordan, the volatile oil of Jordanian S. persica stems exhibited a potent antibacterial activity against both Gram-positive and Gram-negative bacteria [16]. More recently, the activity of aqueous and methanol extracts of IraqiS. persica against seven isolated oral pathogens was tested, andthe strongest antibacterial activity was observed using the aqueous extract against S. faecalis (GI zone: 22.3 mm) [17]. These findings together with the findings of present study strongly suggest that S. persicais a rich source of phytochemicals that have antimicrobial activity, and future studies may go further to isolate and characterize these constituents and test their effectiveness against different pathogens.

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