

The antimicrobial effect of aqueous and ethanol extracts of stems, roots and leaves of *Salvadora persica* on oral microbes

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Abstract:

Fresh samples of *Salvadora persica* were collected from Hudiadah province at 23/2/2011 . They include stems ,roots, and leaves . The ethanolic and aqueous extracts were prepared as w/v . (2.5, 5, 10 %) for three plants parts .

The microbial samples were collected from privet dentists clinics in Tamar province by mouth swab and mouth washing methods . *S. aureu s S. mutans*, , and *C. albicans* were isolated and a pure cultures were prepared to each microbes for farther studies .The effect of different parts of plant showed high antimicrobial activity of root extract followed by stem and leaves extracts and the antimicrobial effecacy concern positively with concentration. The ethanol extract showed more antimicrobial activity than hot water extract.

Introduction:

The current alternative medicine give good evedinces about the trust of using plants as antimicrobial agents and drugs. The WHO reported that 80% of world major part of the therapies involve the use of plant extract or their constituents⁽¹⁾.

Salvadora persica L. (Salvadoraceae) is one of these plants, which was used by Bablonians some 7000 years ago⁽²⁾. According to World Agro Forestry Center(www.Agroforestrycenter.org .), *S. persica* is an evergreen shrub or small tree known as siwak or arak. Arak trees are widely spread in African, Asian & Middle east countries such as Algeria, Egypt, Ethiopia, Libya, Nigeria, India, Jordan, Oman, KSA, Syria and Yemen.

In general the plant prefers areas where ground water is readily available⁽³⁾. The plant is natively to republic of Yemen, especially in Al-Hodeida governorate.

Oral hygiene by using siwak stick is a part of Islam regime⁽³⁾. Sticks of siwak were prepared from roots or shoots and used as toothbrush⁽⁴⁾. Siwak sticks are useful physically due to their fibers and phytochemicals for their antimicrobial efficacy⁽⁵⁾.

Water, ethanol, methanol, ethyl acetate and acetic acid were used as solvents to extract the effective chemical compounds from siwak sticks^{(6),(7)}. The chemical compounds isolated

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from root sticks are 3benzel-isothiocyanate, saponins, tanins, silica, small amount of resin, trimethylamine and fairly large amount of alkaloidal constituents⁽⁸⁾. Lewis and Elvin-Lewis (1977)⁽⁹⁾ reported a high content of minerals in the roots.

In vitro studies showed the antibacterial effects of many extractions from siwak sticks⁽⁵⁾. Aqueous extract has a fungistatic effect on *C. albicans*⁽¹⁰⁾. Al-Ali and Al-Lafi (2003)⁽¹¹⁾ reported that the leaves have benzyl nitrate and the stem has cineol as a main components. Anti-plasmodia effect of *S. persica* was reported by Ali *et al* (2002)⁽¹²⁾.

Clinical studies in children have evaluated the efficiency of siwak as an oral hygiene tool among various population and find to be effective in removing oral deposits⁽¹³⁾.

Although Arak is a well-known shrub in Yemen but the plant has not received much attention. The aim of this study is to detect the antimicrobial activity of aqueous and ethanol extracts of leaves, shoots and roots of native *S. persica* against oral microbes.

Materials and Methods

1. Microbial samples :

Fifty oral swabs were collected from patients of privet dental clinics in Tamar city in addition to ten normal samples. Mouth washing samples were collected to test the effect of plant extracts against oral microbes.

The swabs were cultured directly by streaking method on nutrient agar plates (90mm), then were incubated at 37C°. The growing microbes were isolated, purified and identified following standard methods⁽¹⁴⁾. The yeast pure cultures were prepared on nutrient agar (NA) with chloramphenicol (250 mg/l) to avoid bacterial growth. Germ tube test was followed to identify *Candida albicans*⁽¹⁵⁾.

Sterile distal water (10 ml) was used to prepare mouthwash samples which collected in sterile vials with wide mouth. Mouth-washing samples were used to explain the inhibitory growth effects of water extracts of root, shoot, and leaves on total microbes by colonies count method.

Plant extracts :

Arak samples were collected from Al-Hodeida province, they include stems and roots sticks besides the leaves. Samples were washed intensively by tap water and were dried at room temperature for two days⁽¹⁶⁾.

The samples were grounded by household grinding machine. The powder of each plant parts were used to prepare 2.5%, 5% and 10% (w/v) of boiling distal water and ethanol as a solvents. The plant extracts were purified by fine mish then sterilized by millipore filter papers 0.45µm.

The sterile extracts were used to prepare nutrient broth media in sterile test tubes. The media were inoculated by full loop of pure culture of *S. aureus*, *S. mutants*

and *C. albicans*. After incubation at 37C° microbial growth were tested in the test tubes indirectly as turbidity by spectrophotometer. Test tube with nutrient broth only was used as a control.

Hole plate diffusion method was followed to show the inhibition zones of each solvent extract against microbes. Ethanol extracts was prepared as in aqueous extracts.

The Result and Discussion

Table-1/ The percentage of microbial Occurrence in the samples.

The microorganism	Occurrence%*
<i>Staphylococcus aureus</i>	82
<i>S. mutans</i>	60
<i>Candida albicans</i>	94

*Occurrence%= (no. of samples with microorganism/50)x100

According to the colonies count test(Fig-1), the recent study showed that roots extract give the highest inhibition zone for total microorganisms followed by shoot and leaves extractions. This results were confirmed by test of the microbial growth in broth

All collected samples showed microbial growth on nutrient agar plates. Patient's swabs and mouth washing samples showed more intensive microbial growth than normal swabs.

Two species of bacteria and one yeast were isolated and identified. They were *Staphylococcus aureus* , *S. mutans* and *Candida albicans*. The three microbes are common in the samples.(Table-1)

medium, the highest turbidity (lowest absorption)-measured by spectrophotometer- was recorded when leaves extract was used to prepare the growth medium followed by shoots and roots extracts.

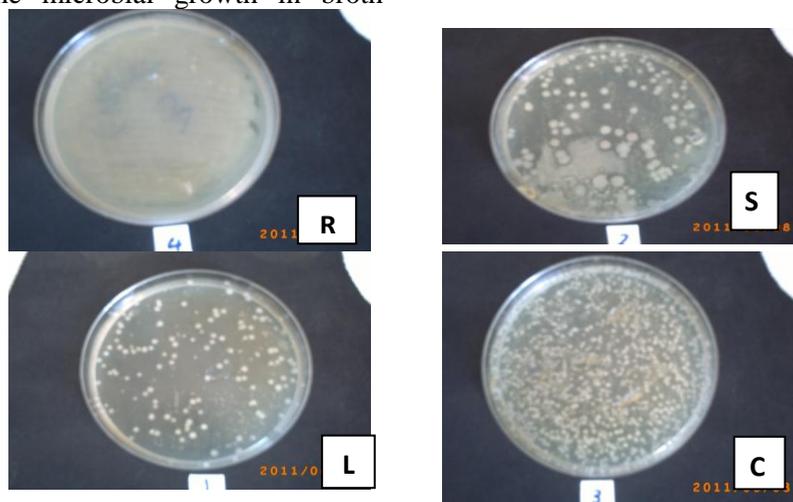


Fig.1/Effect of different plant parts on total microbial growth(R/root), S(stem), L(leaves), C(control).

Different compounds and their concentrations in roots, shoots and leaves may cause different inhibition levels. The extract of root showed the highest antimicrobial effect(fig.1-R) .It contain benzylisothiocyanate which is reported to have a broad spectrum bactericidal activity⁽²⁰⁾ and is the most potent⁽⁶⁾ while stem extract gave a moderate effect(fig.1-S)

Leaves extracts inhibit microbial growth mildly in compare with roots and shoots extracts(fig,1-L), they have benzyl nitrate as the main oil compounds which exhibit antibacterial activity⁽¹¹⁾. The World Agroforestry Center (WAC) reported

that decoction of leaves are used as mouthwash, and masticated leaves for tooth and gum problems⁽²¹⁾.

To compare between ethanol and hot water extract on growth of isolated microbes ,plant extracts were used to prepare the culture media . The results showed that the ethanolic extract has highest antimicrobial activity than aqueous extracts against bacterial and yeast isolates (fig.2) Such effects may be related to several chemical compounds found in siwak sticks include trimethylamine, salvadorine (alkaloids), chlorides, fluorides, sulfur, tannins, flavenoids and sterols⁽¹⁾.

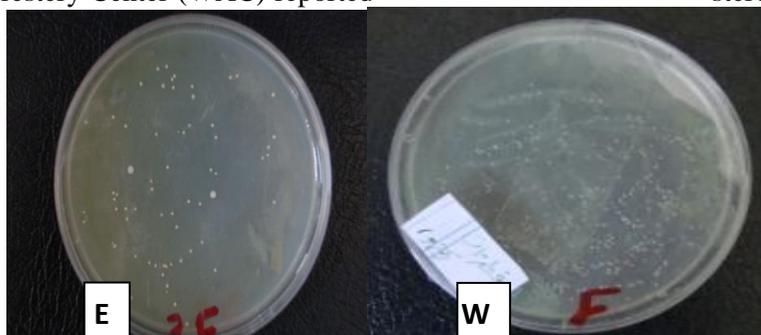


Fig.2- Effect of ethanolic extract (E) and water extract (W) on growth of candida.

Alma(1993)⁽¹⁷⁾ suggested that alkaloids present in *S. persica* is salvadorine which yield trimethylamine on hydrolytical cleavage, these compounds beside sulfur which present in siwak also have bactericidal effects^(9,18).

The effect of aqueous extract of siwak against *C.albicans*

which proved in current study agree with Al-Bayati and Suliman (2007)⁽²⁾. Tannic acid may caused such effect on *C. albicans*⁽³⁾⁽¹⁹⁾.

Ethanol extracts showed more effective zones than water extracts of *S. aureus*, *S. mutans* and *C.albicans* (Table-2) .

Table -2/ The inhibition zone(mm.) of root(R),stem(S),leaves(L) extracts according to solvent and concentration.

Plant extract	Conc.%	<i>S.aureus</i>			<i>S.mutans</i>			<i>C.albicans</i>		
		R	S	L	R	S	L	R	S	L
Ethanol ext.	2.5	4	3	1>	6	4	1>	4	2	1>
	5	10	6	3	11	5	3	7	3	1>
	10	13	8	3	18	7	3	11	7	4
Aqueous ext.	2.5	2	1>	1>	3	1>	1>	1	1>	1>
	5	5	2	2>	7	3	1>	5	2	1>
	10	8	5	3	12	7	2	6	3	2

Al-Lafi and Ababneh (1995)⁽⁵⁾, by using water extract reported(20mm and 24mm) as an inhibition zones for *S. aureus* and *S. mutans* ,respectively the differences from the present study may due to the degree of siwak extract concentration or/and related to different bacterial isolates.

The solvents may give different results also do the age of plant parts used in preparing the extracts. In this study fresh parts (un stored) were used. Alcoholic

extracts showed considerable effects, which increase with extract concentration for roots, shoots and leaves, these results agree with^{(5), (6), (22)}.

In all tests followed here, the inhibition effects of extracts against microbial growth are increase from 2.5%, 5% and 10% respectively (Table-2) . Turbidimetry method and inhibition zones give the same results which agree with previous studies⁽⁵⁾.

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التأثير الضد ميكروبي للمستخلصين المائي والايثانولي لساق وجذر وأوراق

نبات الأراك *Salvadora persica* على ميكروبات الفم

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جمعت عينات نبات الأراك الطازجة من مناطق نموها الطبيعية في محافظة
الحديدة – اليمن بتاريخ 23-2-2011 م واشتملت العينات على قطع من الساق
والجذر وكذلك جمعت الأوراق الخضراء وحُضِر من جميع العينات التي تم جمعها
المستخلص المائي الساخن والايثانولي بتركيزات (2,5 % ، 5 % و 10 %)
وزن / حجم.

أخذت العينات الميكروبية من أشخاص يراجعون العيادات الخاصة بأمراض الأسنان
في محافظة ذمار وكان الجمع بطريقتي المسحة وجمع ماء المضمضة . عزلت
ونقيت مزارع *S. aureus* و *S. mutans* و *C. albicans* لأجراء الاختبارات
عليها والتي شملت اختبار تباين التأثير المضاد للميكروبات بحسب الجزء النباتي
المستعمل لتحضير المستخلص واختبار تأثير اختلاف التركيز على الفعالية المضادة
للنمو الميكروبي ، ، وكذلك المقارنة بين المستخلصين الكحولي والمائي الساخن .
بينت النتائج بان مستخلص جذور الأراك فاق في تأثيره المثبط للنمو الميكروبي
مستخلصي الساق والأوراق على التوالي كذلك أظهرت النتائج ان زيادة التأثير
المثبط للنمو للمستخلصات المائية للجذور والساق والأوراق تزداد مع زيادة
تركيزها، وكان المستخلص الكحولي ذو فعالية أعلى من المستخلص المائي
الساخن.