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The Activity of Salvadora Persica Extracts as Antioxidant and Antimicrobial

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Abstract

Herbal medicine is an effective treatment for many diseases that are intractable to traditional medicine. Salvadora persica L. is an extensively utilized plant by many Arab populations worldwide due to its varied medical benefits. The study looks at plant roots' antimicrobial and free radical scavenging properties of flavonoid, glycoside, and alkaloid extracts. The activity of the extracts was evaluated for Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. Based on the findings, the three extracts recorded no activity towards the selected microorganisms except the flavonoid extract which showed an efficacy close to that of amoxicillin against Staphylococcus aureus and Escherichia coli growth with inhibition zones 14mm and 15mm respectively. Inhibiting lipid peroxidation was more successfully accomplished by flavonoid extract which recorded 50% compared to standard compound BHT (60%), the glycoside and alkaloid extracts exhibited noticeable activity as antioxidant with 27% and 18.4 % inhibition.

Keywords: Salvadora persica, Miswak, Antimicrobial, Antioxidant, Flavonoids.

1.Introduction

Salvadora persica L. (family: Salvadoraceae), also known locally as miswak, toothbrush tree, and peelu is an old native plant that may be found in nations all over the world, including Oman, Yemen, Saudi Arabia, and Jordan, as well as, Sri Lanka, Pakistan, India, and Iran. A mushy pink fruits, slightly rough bark, large crown of curving branches, a stem that is greyish-brown, and greenish-yellow blooms are all features of this evergreen tree [1-3]. one of the plant's principal traditional uses is using the root as stick for cleaning teeth which is an ancient widespread custom. As a useful tool for maintaining dental hygiene, chewing sticks have received recommendations and encouragement from the World Health Organization and miswak is the most widely used of the 182 plant species appropriate for making toothbrushing sticks[4,5]. Other use for the plant include the treatment of gonorrhea, ulcers, chest diseases, stomachaches, headaches, spleen issues, and boils. Using the root drink can help mothers produce more milk [6]. Bark latex of plant is useful for healing skin wounds. The plant's leaves are also used for treatment of cough, piles, piles-related pain, stomachache, tooth disorders, bodily pain, and to cure wounds. There is a strong belief that the honey from S. persica has a high therapeutic value

and that honey bees find the flowers to be a rich source of nectar. The seeds are consumed as a tonic, and the seed oil is applied topically to cure rheumatism, lumbago, and joint discomfort. Additionally, it has been used to treat worms, malaria, fever, and edema. Women utilize the plant juice as a female contraceptive [6-8]. Many studies reported that S. persica's portions contain a variety of bioactive organic and inorganic substances: alkaloids, terpenes, saponins, beta-sitosterol and glucosides, cyanates, amides, pyrrolidine, pyrrole and piperdine derivative, resin, salvadorine, tannins, terpenoids, sterols, salvadourea, flavonoids, and vitamin C, and the fatty acids (stearic, linoleic and oleic acids) [9]. Also the plant contain Na, Ca, S, K, P, F, Mn, Mg, Si, NaCl, and KCl [10-12]. According to these diverse compounds, the plant has many biological activities. Many studies demonstrated the anti-microbial activity of Salvadora persicais against pseudomonas aeruginosa, lactobacillus, bacillus subtilis., staphylococcus aureus, aspergillus niger, streptococcus mutans, candida albicans, Escherichia coli, proteus valgaris and lactobacillus brevis. Additionaly, this plant has been utilized in many fields, including anticancer, wound curing, radical scavenger, antiulcer, anticonvulsant, antifertility, anti-inflammatory and hypolipidemia properties. Also, the plant is used in food, cosmetic and fuel fields [13-15]. The aim of this study is determination the activity of flavonoid, alkaloid and glycoside extracts of miswak root as antimicrobial and antioxidant.

2.Materials and Methods

2.1 Plant material

The miswak sticks used in this investigation were purchased from a medicinal herb market. The classification of plant was carried out by biology department in sciences college as described as follows: Kingdom: Plantae. Class: Magnoliopsida. Order: Brassicales. Family: Salvadoraceae. Genus: Salvadora. Species: Salvadora Persica L. The plant was taken and pulverized, and the powder was kept until it was required.

2.2Reagents

Ethyl alcohol, methyl alcohol, lead acetate, HCl, acetone, ethyl acetate, acetic acid, sulphuric acid, ammonium hydroxide, chloroform, n-butanol, sodium chloride, DMSO, β –carotene, linoleic acid, Tween 20 and BHT. All of the reagents and solvents used were acquired from Sigma-Aldrich and are of the highest quality possible.

2.3Ethyl alcohol Extract

5 grams of crushed miswak root were mixed with 50ml of 70% ethanol in room temperature for 12hours. The precipitate was removed by filtering and the filterate used for the preliminary qualitative testing measurements [16].

2.4 Qualitative phytochemical screening

Phytochemical analysis of S. persica extract was done to identify its various components such as; flavonoids, tannins, proteins, amino acids, glycosides, alkaloids, triterpenes, and saponins.

2.5 Extraction of Flavonoids(F):

17gm of Miswak mixed with 200 ml of 70% methyl alcohol and stirring at room temperature for 24 hours. Then, the extract filtrate mixed with 1% aqueous lead acetate. The formed precipitate was split up by filter paper and treated with (10 mL 2N HCl and 50 mL acetone), again the mixture filtered and the filterate dried in room temperature to get about 0.1gm of amorphous powder which diluted in water (25 ml) and extracted by ethyl acetate (2x25ml), the collective ethyl acetate parts were dried to afford 0.2gm(the yield 1.2%)[17].

2.6 Extraction of Alkaloids(A):

On a water bath, about 17grams of miswak were mixed with 250 ml of (ten percent acetic acid in EtOH) for 24 hours. Then, the filtrate was concentrated till 15mL and a few drops of 2% sulphuric acid was added. Then, the filtrate basified till arriving to pH 9 with ammonium hydroxide and extracted with 25 ml chloroform for 3 times. The collective chloroform parts were evaporated and gave about 0.1gm(the yield 0.6%)[[18].

2.7 **Extraction of Glycosides(G):**

17 grams of dried miswak were admixed with 150 ml of 2% acetic acid, heated for 8 hours over a water bath while stirring, filtered, and the filtrate was extracted using n-butanol that had been pre-saturated with sodium chloride [19]. 0.15 gm was obtained by evaporating the butanol portion in air(the yield 0.9%).

2.8 Antimicrobial Activity:

Antimicrobial properties of the extracts were tested in vitro using agar well diffusion assay. Staphylococcus aureus as Gram-positive bacterium, Pseudomonas Aeruginosa and Escherichia coli as Gram-negative bacteria, Candida albicans was a fungal pathogen. On the surface of Nutrient agar N.A medium and Sabouraud dextrose agar (S.D.A) medium, 0.2 mL of bacterial and fungal inocula were positioned, respectively. DMSO was used to dissolve the samples at a concentration of 0.03g/mL used for the extracts and reference medication. The samples were inserted in central pore, fungal plates were incubated at (25± 2°C) for 24 hours whereas bacterial plates incubated at (37 ± 2°C) for 24 hours [20]. Inhibition zones measured in millimeter units.

2.9 Determine the Antioxidant activity of extracts

The anti-oxidation activity of the three extracts was evaluated using the oxidative losses of a -carotene/linoleic acid emulsion. Tween 20 and linoleic acid were mixed in a round flask along with 1 ml of -carotene (0.2 mg/ml in chloroform). 50 ml of distilled water was added after the chloroform had evaporated, and 3.8 ml of this mixture was dosed with 0.2 ml of the test sample and the reference material (butylated hydroxyl toluene, or BHT), with the absorbance at 470 nm

being recorded. After that, the samples were heated to 45 °C for two hours in a water bath, and the absorbance was measured every 15 minutes [21]. To calculate antioxidant activity (AA), the following equation was used:

$$\%AA = 100\{1 - [Ai - At / *Ai - *At]\}$$

Where Ai: initial sample absorbance, At: final sample absorbance after (105)min, *Ai: initial control absorbance, *At: final control absorbance after (105)min.

3. Result and Discussion

3.1 Extraction and phytochemical screening

The plant material was extracted using 70% ethanol which gave a percentage yield of 24 %. Then in the phytochemical screening of the dried root of miswak it revealed the presence of flavonoids, tannins, glycosides, amino acids, alkaloids, saponins, terpenoids, and carbohydrates and absent the protein [The results could be seen in Table 1].

Table(1): Preliminary Tests on the Ethanol Extracts.

Active Part	Reagent	Result
Alkaloids	**Dragendorff**	**+**
Flavonoids	**Ethanolic Potassium Hydroxide**	**+**
Glycosides	**Molish Test**	**+**
Proteins	**Biuret Test**	**-**
Amino Acid	**Ninhydrin Test**	**+**
Saponins	**Mercuric Chloride**	**+**
Tannins	**-Ferric Chloride** **-Lead Acetate**	**+** **+**
Triterpenoids	**Chloroform+Sulfuric Acid**	**+**

3.2 Antimicrobial Activity

The well agar diffusion method used to evaluating the antibacterial and antifungal action of the studied extracts against some pathogens (Ps. Aeruginosa, E.coli, S. aureus and C.albicane). The results are presented in Table 2. When compared to the standard drug amoxicillin, the flavonoid extract had good activity against some microbes, with inhibition zones of 14 and 15mm for S. aureus and E.coli, respectively, with no activity towards C.albicane. The glycoside and Flavonoids are the most common type of polypehol, and they have a variety of biological functions comprising antibacterial, anti-inflammation anti-oxidation, anti-cancer, and cardiovascular protection[16]. Flavonoids' antibacterial action against Gram-negative and Gram-positive bacteria is becoming more well-known. Many research groups have screened crude plant extracts with a history of folk medicinal use for antibacterial activity. This activity can be carried out in three means: reducing bacterial pathogenicity, directly killing bacteria, and

activating antibiotics synergistically. There are two mechanisms to work when flavonoids interact with bacterial lipid bilayers. The first is linked to the partition of non-polar chemicals in the membrane's hydrophobic interior, while the second is linked to the formation of hydrogen bonds at the membrane interface between the more hydrophilic flavonoids and the polar head groups of lipids [16-18]. alkaloid extracts had no activity against studied microbes.

Variations in the rate of penetration within the cell wall and cell membrane architecture of bacteria could explain the disparities in susceptibility to extracts among the test species[19].

Table(2): The Activity of Extracts as Antimicrobial

Sample	S. Aureus	E.Coli	Ps. Aeruginosa	C.Albicane
A (Alkaloid)	0	0	0	0
F (Flavonoid)	14	15	0	0
G (Glycoside)	0	0	0	0
Amoxicillin	12	15	45	---
Nystatin	-----	----	----	35

3.3 Antioxidant Activity

The antioxidants activity of the three extracts was established using the β –carotene bleaching process, which was constructed on the relationship between absorbance and time as presented in Table 3 and figure1, and compared the results to BHT, that used as a standard due to its phenolic structure and has been used in several food systems, the results demonstrated that the highest percent reduction of lipid peroxidation belong to flavonoids extract which was 50% compared to reference BHT (60%), this is due to the presence of excess phenolic hydroxyl groups, which might rise antioxidant activity[20]. In addition, the glycoside extract has a moderate antioxidant activity (27%) compared to BHT, however the alkaloid extract has a lower antioxidant activity (18.4%). Flavonoids have the ability to prevent and quench the production of reactive oxygen species (ROS) [21], which are created by external harm or caused during normal oxygen metabolism and causing damage to body cells and tissues. One way that free radicals appear to disrupt biological processes is by lipid peroxidation, which damages cellular membranes. Due to the cellular damage, the net charge of the cell changes, changing the osmotic pressure, resulting in swelling and ultimately cell death. Reactive oxygen species can be avoided by living organisms by using a variety of effective defense mechanisms[22]. Flavonoids are oxidized by radicals, producing a more stable, less reactive radical as a result. Radicals are rendered inactive by the strong reactivity of the hydroxyl groups in flavonoids. According to the following equation, the radical molecule will get a hydrogen atom from the free hydroxyl group, which will stabilize it and produce a relatively stable flavonoid phenoxyl radical [22,23]. Flavonoid(OH) + R• = flavonoid(O•) + RH

Also , the antioxidant activity of the alkaloids and glycosides have been demonstrated in several studies [24-27].

Table(3): Effectiveness of extracts as antioxidants compared with BHT

Sample	Ai	At	*Ai	*At	Aa%
Bht	2.436	2.364	1.73	1.559	60
A(Alkaloid)	2.135	1.988	1.73	1.559	18.4
G(Glycoside)	1.946	1.813	1.73	1.559	27
F (Flavonoid)	1.756	1.665	1.73	1.559	50

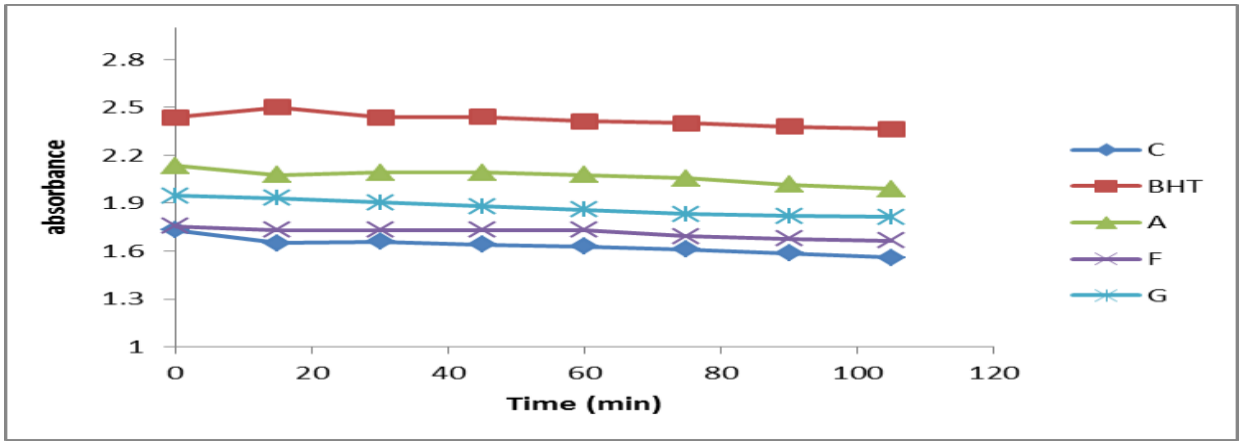

Figure. (1) Antioxidant action of glycoside (G),flavonoid (F) and alkaloid (A) extracts.

4. Conclusions

The results show that the flavonoid extract of Miswak has good antibacterial activity against gram negative and gram positive bacteria, indicating that the extract could be used as a natural antibiotic. Additionally, the three extracts of miswak, particularly the flavonoid extract, showed antioxidant activity, indicating that they could be used as natural antioxidants for medicinal and commercial purposes.

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