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Study properties the active and biological compounds of the alcoholic extract of roots Salvadora persica and inhibitory effect for microorganisms

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Abstract

The study aimed to investigate the active and bioactive compounds and study the chemical composition of roots of Salvadora persica, as well as the inhibitory effect of alcoholic extracts on microorganisms. The results showed that the chemical composition of the Salvadora persica roots contained moisture, ash, protein, fat and carbohydrates amounting to 64.33%, 10.08%, 6.92%, 2.21% and 16.46%, respectively. Qualitative analysis the active compounds of aqueous extract of Salvadora persica showed that it contained saponins, phenols, alkaloids, steroids and tannins. GC-MS analysis of the aqueous extract of Salvadora persica revealed 20 active compounds, the most important of which were Isothiocyanic acid and 2,5-Dimethyl-3,4 dihydrofuran. Analysis of the alcoholic extract of Salvadora persica revealed that it contained 18 active compounds, the most important of which were 6-Octadecenoic acid, Isothiocyanic acid and benzylester.

Keywords: Salvadora persica, alcoholic extracts, microorganisms, isothiocyanic

1. Introduction

Studies have proven that Salvadora persica has a significant effect on reducing the growth of bacteria in the mouth, as it contains active antibacterial substances including sulfur and triamine, which work to lower the pH of the mouth and thus prevent bacterial growth. It also contains vit C and citrulline, both of which play an important role in protecting the gums from inflammation. fluoride, chloride, and silica are known to whiten teeth, The extract of Salvadora persica led to a

statistical decrease in the bacterial count of staphylococcus bacteria that cause caries, as well as bacteria that cause gum disease. It was also found that the raw Salvadora persica had a more effective effect in reducing bacteria than the aqueous extract[8].In addition to these compounds the chemical analysis of the extract showed that it contains sulfur and isothiocyanide compounds responsible for the inhibitory effect of extract against microbes and trimethylamine reduces adhesion to surfaces and removes accumulated bacterial plaque and Tannins isothocyanate, Tannic acid and Benzyl have a major role in the inhibitory effect against some microorganisms and in treating infections in the gums[14].

2. Materials and Methods

A.Sample Collection

The roots of Salvadora persica were harvested, cut, dried and ground using an electric grinder to obtain a fine homogeneous powder. These powders were stored in clean sterile glass bottles until use.

B.Chemical Analysis of Salvadora persica root powder

1.Moisture Estimation

1 gram of Salvadora persica powder was taken and dried in a convection oven at 105°C until dry and the weight was constant. The sample was placed in a drying vessel to prevent moisture from accumulating and to achieve a constant weight[1].

2.Ash Estimation

1 gram of sample was taken the empty vessel was weighed and the sample was burned over a flame. It was then placed in an incineration oven at 550°C for 7 hours until white ash was formed[1].

3.Protein Estimation

The percentage of nitrogen was estimated using the standard microkjeldahl method by digesting 0.2 gm of powdered Salvadora persica roots followed by distillation using a Vapodes 45S distillation system attached to the device and sieved using an auto-sieving unit. The protein percentage was calculated by multiplying the total nitrogen percentage by the general protein coefficient of 6.25[1].

4.Fat Estimation

The fat percentage was estimated according to the method described[1] using a Saxolite apparatus. 10 gm of the sample was placed in a cellulose extraction thimble. Hexane was used at a temperature of 40-60°C in the extraction process, which took 6-8 hours. The solvent was then evaporated using a rotary evaporator under vacuum pressure at a temperature of 60°C.

5.Carbohydrates Estimation

The percentage of carbohydrates in the powdered Salvadora persica roots was estimated according to the established method[1].

C. Chemical detection of some active compounds in the roots of the Salvadora persica

The active ingredients in the roots of the Salvadora persica were detected according to the method described previously[16].

1.Detection of phenols

Phenols were detected by adding 3 ml of Salvadora persica root extract to 2 ml of 1% ferric chloride solution. The appearance of a dark green color indicates the presence of phenols.

2.Saponins

Saponins were detected by adding 5ml plant extract to a graduated test tube and

shaking the tube for 15 minutes. The appearance of 2ml thick foam indicates the presence of saponins.

3.Steroids

Steroids were detected by adding 2 ml of Salvadora persica root extract to 10ml chloroform. Add 1 ml of anhydrous acetic acid, then add 2ml of concentrated sulfuric acid. The appearance blue or green color indicates the presence of steroids.

4.Alkaloids

Alkaloids were detected by boiling 10 grams of Salvadora persica root extract with 50 ml of distilled water containing 4% hydrochloric acid. 0.5 ml of the filtrate was taken and several drops of Mayer's reagent were added to the tube. The appearance of a white precipitate indicated the presence of alkaloids.

5.Tannins

Tanins were detected by adding several drops 1% lead acetate solution. The appearance of a white precipitate indicated the presence of tannins.

D. Analysis the active compounds using GC-MS for the alcoholic extract of Salvadora persica roots

The active compounds of alcoholic extract of the roots of the Salvadora persica were detected using a gas chromatography analyzer connected to a mass spectrometer equipped with an electronic computer. The mass spectrum of active compounds present in the plant extract was measured at the University of Nahrain / Baghdad in the Biotechnology Research Center. 1gm of extract was taken and placed in a test tube and 1 ml of methanol, 1ml of 0.1N potassium hydroxide and 10ml of heptane were added to it. Then the tube was shaken with its contents for twenty seconds and placed in GC-MS device and the results appeared[6].

E. Estimation the inhibitory activity of Salvadora persica root extract

The inhibitory activity of the roots of the Salvadora persica was estimated according to method described by[9]. The method of diffusion in holes was used to study the effect of aqueous extract for Salvadora persica roots on bacteria. The agar medium nutrient for the test bacteria was inoculated by spreading 0.1 of bacterial suspension containing 1.5×105 cells/ml compared to McFarland's solution. Holes with a diameter of 5 mm were made on surface the medium using a cork drill. The prepared concentrations of the aqueous extract of the Salvadora persica powder were placed at concentrations 0.25, 0.50 and 1mg/ml, respectively. Then 0.1 ml of the concentrations were taken and placed in the hole. The plates were left to solidify, after which these plates were incubated for 24 ± 2 hours in the incubator at a temperature of 37°C. The diameter of the inhibition zone around each hole was measured.

3. Results and discussion

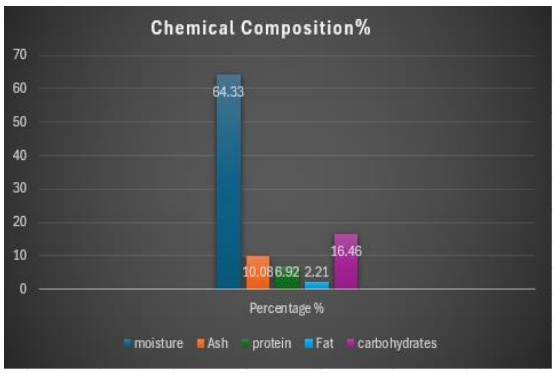
1.Chemical composition of the Salvadora persica root powder

The results in Table(1) show the percentages of chemical components of the dried Salvadora persica root powder with the moisture content being 64%, ash content 10.08%, protein 6.92%, the fat 2.21% and carbohydrate16.46%. These results were similar to what was found by[3]. When studying the chemical content of Salvadora persica roots the moisture content was 65.31%, ash content 9.5%, protein 6.28%, fat 2.12%,

and carbohydrate was 17.23% on a wet weight basis. As shown in figure(1).

Table(1):Chemical Composition of Salvadora persica Root Powder

Component	Percentage %
Moisture	64.33
Ash	10.08
Protein	6.92
Fat	2.21
Carbohydrates	16.46



Fig(1):Chemical Composition of Salvadora persica Root Powder

2.Qualitative chemical detection of some active compounds in an extract of Salvadora persica roots.

The results in Table(2) showed that the hot aqueous extract of the Salvadora persica contained various active compounds through chemical analyses that yielded positive results for each of the active compounds, namely saponins, phenols, alkaloids, steroids and tannins through the appearance of different colors specific to each type, which indicates the result of the detection of these compounds that were consistent with[15], when detecting these active compounds in the roots of Salvadora persica.

Study[7] found that the roots of Salvadora persica contain active compounds, including phenols, steroids, alkaloids, saponins, tannins, glycosides and flavonoids.

The wolf grape plant contains active compounds such as alkaloids, saponins, resins, tannins, coumarins and glycosides which are found mainly in the fruits and leaves to a lesser extent in the roots which don't contain glycosides. A study[4] showed the absence of steroids and alkaloids in the leaves of the eucalyptus plant.

Table(2):Phytochemical Screening of hot aqueous extract of Salvadora persica roots

Phytochemical group	Result
saponins	+
phenols	+
alkaloids	+
steroids	+
tannins	+

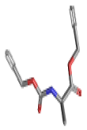
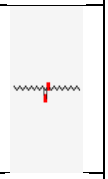
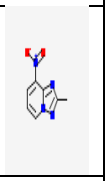
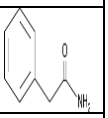
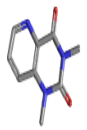
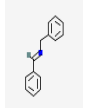
3.Estimation of the active compounds of the alcoholic extract of Salvadora persica roots using GC-MS technology

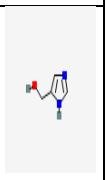
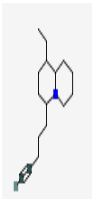
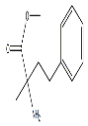
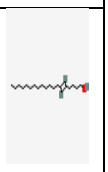
The results at Table(3) indicated that the alcoholic extract of Salvadora persica roots contained eighteen active compounds which were estimated using a gas chromatography device connected to a mass spectrometer. The results of the chromatography analysis showed the presence of the compound 6-Octadecenoic acid at a higher percentage of the compounds, reaching (32.31)%.

Then followed by organic acid Isothiocyanic benzyl ester acid at (23.42)%. The analysis results showed the presence of Hexadecanoic acid (16.66)%. It also showed the presence of 3-Methyl-1-propyl-4,5-dihydro-1H-pyr(4.49)%. It showed the presence of 5-Nonadecen-1-ol (4.07)%.The compound 2-hexyl-methyl

ester was also found(3.16)%.alpha.-1-(Ethylmethy lamino)ethylbenzenemethanol(2.24)%. Also,the presence of 4-(Hydroxymethyl)imidazole(2.01)%. The presence of 2-Amino-2-methyl-4-phenylbutanoic acid was also observed(1.88)%. The analysis also showed the presence of methanol 1H-Imidazole-4-methanol(1.83)%.[11] Maleinimide was observed(1.44)%.The analysis revealed the presence of alpha.-Toluamide(1.22)%.The compound (4R,6S,9aR)-4,6-Di(pent-4-en-1-yl)octahydro-1H-quinolizine was found(0.99)%. The compound 1,3-Dimethylpyrido(3,2-d)pyrimidine-2,4 (1H,3H)-dione was observed(0.93)%. The compound undecyl ester was also found(0.91)%. The compound Benzylamine, N-benzylidene was observed at a rate of (0.89)% and the compound,propargyloxycarbonyl(0.89%. The compound s-Triazolo[1,5-a] pyridine, 8-nitro was found(0.66)%.These compounds have an impact on biological activity and antioxidant activity because they lead to the suppression of free radicals and the inhibition of living organisms. The results of a study by[10], appeared when analyzing the extract of the Salvadora persica which found the highest percentage of compound 6-Octadecenoic acid and it agreed with what was mentioned by[5], when he studied the chemical composition of the Salvadora persica.

Table(3):Bioactive compounds alcoholic extract of Salvadora persica roots by GC-MS analysis.

n	Identified Compounds	Molecular Weight	Percentage	Molecular Formula	Chemical Structure
1	n-propargyl oxycarbonyl	209.2	0.89	$C_{10}H_{11}NO_4$	
2	undecyl ester	354.6	0.91	$C_{23}H_{46}O_2$	
3	3-Methyl-1-propyl-4,5-dihydro-1H-pyr	84.1	4.49	$C_4H_8N_2$	
4	s-Triazolo[1,5-a]pyridine, 8-nitro	178.1	0.66	$C_7H_6N_4O_2$	
5	Isothiocyanic acid, benzyl ester	149.2	23.42	C_8H_7NS	
6	alpha.-Toluamide	135.1	1.22	C_8H_9NO	
7	1,3-Dimethylpyrido[3,2-d]pyrimidine-2,4(1H,3H)-dione	191.1	0.93	$C_9H_9N_3O_2$	
8	Benzylamine, N-benzylidene	195.2	0.89	C14H13N	
9	alpha.-[1-(Ethylmethylamino)ethylbenz	193.2	2.24	C12H19NO	

	enemethanol				
10	Hexadecanoic acid	256.4	16.66	C16H32O2	
11	6-Octadecenoic acid	282.5	32.31	C18H34O2	
12	2-hexyl-, methyl ester	322.5	3.16	C21H38O2	
13	Maleinimide	97.0	1.44	C4H3NO2	
14	4-(Hydroxymethyl)imidazole	98.1	2.01	C4H6N2O	
15	(4R,6S,9aR)-4,6-Di(pent-4-en-1-yl)octahydro-1H-quinolizine	233.3	0.99	C16H27N	
16	2-Amino-2-methyl-4-phenylbutanoic acid	207.2	1.88	C12H17NO2	
17	5-Nonadecen-1-ol	282.5	4.07	C19H38O	

4.Testing the inhibitory activity of the Salvadora persica root extract

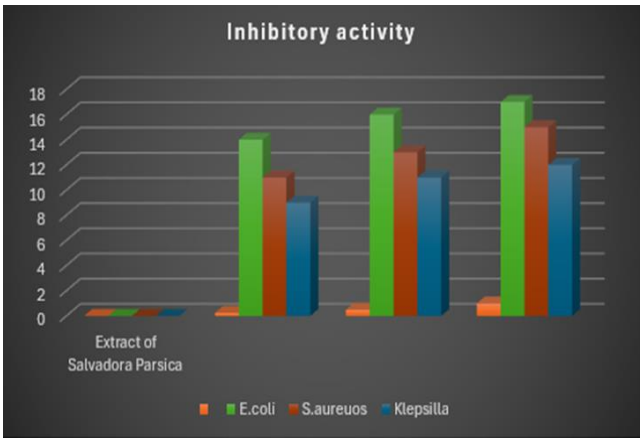
Table(4) shows the diameter of the inhibitory halo(mm) of aqueous extract of Salvadora persica against the types of bacteria used. The study showed a clear variation in the rates of inhibitory activity for the growth of the bacteria used. The aqueous extract of Salvadora persica root showed inhibitory activity against the growth of three types of bacteria, as it recorded the highest inhibitory halo diameter (17)mm against E.coli. As for Staphylococcus aureus bacteria, it was recorded as (15)mm, while the lowest diameter of the inhibition halo was (12)mm for Klepsilla bacteria. These results were consistent with[7], which showed when studying that the use of the Salvadora persica extract led to the inhibition of microorganisms, as the inhibition was (18)mm for E.coli bacteria,(16) mm for S.aureus and (14)mm for Klepsilla bacteria[11]

The higher the concentration of the extract, the greater its effect on bacterial growth due to damage to the cell wall and the proteins and fats it contains. Thus, the microorganisms lose their ability to reproduce or through inhibiting some reactions necessary for growth or for another reason as a result of blocking the active sites of the enzymes present within the microorganisms that are necessary for growth and reproduction[2]. Some studies have shown that the plant contains flavonoid compounds that have activity against Gram-positive microorganisms S.aureus[13]. Extract of Salvadora persica that a strong source of active compounds that contain anti-

inflammatory and anti-microbial1. properties. The results showed that the use of Salvadora persica roots had an effect against microbial infections[12].

Table(4): Inhibitory activity of Salvadora persica root extract 2.

Name of Bacteria / Extract		*E.coli*	*S.aureus*	*Klepsilla*
		Areolar diameter (mm)		
Extract of Salvadora persica plant	0.0	0	0	0
	0.25	14	11	9
	0.50	16	13	11
	1	17	15	12



Fig(2): Inhibitory activity of Salvadora persica root extract

4. Conclusions

The Salvadora persica has a good chemical composition of moisture, ash, protein, fats and carbohydrates in ideal proportions. It also contains active compounds that have a positive effect on the body and it has a natural inhibitory effect through its content of a fair number of bacteria.

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