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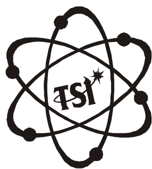

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Salvadora persica: A rich medicinal plant of polyphenols and alkaloids with biological activity

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ABSTRACT

In this study, stems and leaves of *Salvadora persica* were collected from trees grown at Ahaggar in south Algeria. They were powdered and extracted why to assess, the one hand, total phenolic, tannin and flavonoid content and, on the other hand, antioxidant and antibacterial activities of (ethyl acetate fraction, butanolic fraction and alkaloids total). The results showed higher levels of total phenolic, tannin and flavonoid content in leaves (67.32 ± 0.17 and 39.31 ± 3.81 mg PE/g DW and 0.47 ± 0.00 mg RE/g DW). Higher Free Radical Scavenging and iron reducing power of leaves ethyl acetate extract was observed (IC_{50}, 11.8 ± 0.07 µg/ml and 480 ± 6 µg/ml respectively). On the contrary of antioxidant activity, the strongest antibacterial activity of *Salvadora persica* extract was exhibited by Stems Alkaloids extract against *Bacillus cereus* ATCC 11778 (17mm at 20 mg/ml).
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KEYWORDS

Salvadora persica;
Flavonoids;
Alkaloids;
Antibacterial;
Antioxidant.

INTRODUCTION

Salvadora persica is a subtropical tree of medicinal interest, belonging to the family Salvadoraceae, native to the Arabian Peninsula, Egypt, and India[1,2]. This plant is a ever green medium vegetation on saline soils where the salt concentration of the soil would inhibit the growth of most other crops[3]. The species has wide adaptability from sand dunes to heavy soils, nonsaline to highly saline soils, dry regions to marshy and water logged conditions[4]. Almost all plant parts have been found to be pharmaceutically important[5,6].

The most important use of the plant is the use of the stem by muslims as tooth brushes, known as “meswak”. Antibiotic, anti-inflammatory, and mildly hypoglycemic effects of the plant have been reported[7]. It used by traditional health practitioners in Senegal for treatment of respiratory tract[8]. The leaf is also reputed to be diuretic[9]. In Algeria, the stem is used as a tooth-pick. However, the leave is used as a decoction for the treatment of jaundice, aphthae, amenorrhea, gonorrhea and syphilis by folk medicine practitioner[10].

Chemically, the air dried stem bark of *Salvadora persica* is treated with 80 % alcohol and then with ether and other solvents which showed that it is composed of alkaloid may be salvadurine, tannins, saponins, fla-

vonoids and sterols[11]. It has been reported that this plant contains substances that possess antibacterial properties, which encourage some tooth paste manufacturers to incorporate powdered stems material of her in their product[12].

Extracts of this plant have been included as ingredients in oral hygiene products, but despite several studies performed, there is as yet, small data of their effectiveness[13,14].

Although researchers have fixed their work on the antibacterial activity of this plant, must not ignore the other uses (e.g. diabetes). This disease is caused in certain cases of oxidative stress.

It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others[15,16]. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous or endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders[17,18,19]. There is our days, an increasing interest in the measurement and use of plant antioxidants for scientific research[20]. This is mainly due to their strong biological activity, exceeding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis[21].

In fact of the traditional use of *Salvadora persica* stems and leaves, it tried to evaluate the antioxidant and antibacterial activities of alkaloids, ethyl acetate and butanolic fraction of flavonoids, and investigate his polyphenol's, tannin's and flavonoid's content. In Algeria, there is no study on this plant which grows on a large area of Ahaggar.

EXPERIMENTAL

Collection of plant materials

Salvadora persica stems and leaves were collected from plants grown in the region of Ahaggar in south Algeria on 2005, it was identified by Tassili N'Ajjer National Park Algeria and the voucher specimens have been deposited at the Herbarium of Natural Product Laboratory Department of Molecular and Cellular Biology, Tlemcen University, Algeria. They were air- dried and stored in air-tight paper bags until required.

Plant extraction

Salvadora persica stems and leaves were powdered and 0.25 g, 10 g and 10 g of each organ, respectively, was weighted into adequate glass beaker and 10 ml of aqueous acetone (70%), 500 ml of aqueous methanol (80%) and 500 ml of acetic acid in ethanol (10%) were added. The beakers were suspended in a water bath and homogenized with an (ULTRA TURRAX, IKA^R WERKE) at 13500 rpm for 30 min at 4°C. The content of each beaker was filtered separately through filter paper. The residue was again treated with similar manner.

Determination of total phenolic, tannin and flavonoid content

They were determined using extract sample of aqueous acetone because of the higher solubility of tannin and phenolic compounds in aqueous acetone solution, and acetone prevents oxidation of phenols[22].

Determination of total phenolic content

The amount of total phenolic content was determined by Folin-Ciocalteu procedure Singleton and Rossi[23]. Aliquot (0.1 ml) of each sample extract was transferred into the test tubes and their volumes made up to 3 ml with distilled water. After addition of 0.5 ml Folin-Ciocalteu reagent and 2 ml of 20% aqueous sodium carbonate, tubes were vortexed and incubated at room temperature under dark condition. The absorbance was recorded after 1h at 650 nm JEN WAY 6405 UV/Vis spectrophotometer. The total phenolic content was calculated as a Pyrocatechol equivalent (mg PE/g DW), from the calibration curve of Pyrocatechol standard solutions (range 1-15 mg/ml), giving an equation as

Absorbance=0.0132 Pyrocatechol (mg/ml) – 0.035 (R^2 = 0.997)

All tests were carried out in triplicate.

Determination of tannin total content

It was determined by Folin-Ciocalteu procedure Makkar et al.[24] after removal of tannins by their adsorption on insoluble matrix (polyvinylpolypyrrolidone, PVPP). Insoluble, cross-linked PVPP (100 mg) was

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weighed into test tubes and 1 ml of sample extract added to 1 ml of distilled water. After 15 min at 4°C, tubes were vortexed and centrifuged for 10 min at 3000g. Aliquots of supernatant (0.1 ml) were transferred into test tubes and nonabsorbed phenolics determined as described before. Calculated values were subtracted from total polyphenolic contents and total tannin content expressed as a Pyrocatechol equivalent (mg PE/g DW). All measurements were done in triplicate.

Determination of total flavonoid content

It was determined based on the formation of flavonoid-aluminium[25].1 ml of each sample extract was mixed with 1 ml 2% aluminium chloride solution. After incubation for 15 min at room temperature, the absorbance at 430 nm was determined in JEN WAY 6405 UV/Vis spectrophotometer. The calibration curve was performed with Rutin (range 0.1–1 mg/ml), giving an equation as

Absorbance = 2.302 Rutin (mg/ml) + 0,021 (R^2=0.992)

The results are expressed as Rutin equivalent (mg RE/g DW). Tests were carried out in triplicate.

Extraction of flavonoids and total alkaloids

(1) Ethyl acetate and butanolic fractions

Sample extracts of aqueous methanol were evaporated to dry under reduced pressure at 45°C. The dried weight obtained were measured and treated with 10 ml of hot distilled water in order to dissolve flavonoids. Then, they were extracted with ethyl acetate (3x10ml). The remaining extract was continuously extracted with n butanol (3×10 ml). Ethyl acetate extracts and n butanol extracts were washed with dried Na_2SO_4, and evaporated to dryness under reduced pressure at 45°c. The dried weight of each extract were measured and stored at 4°c for further tests[26].

(2) Total alkaloids

The method reported by Harborne[27] was employed. So, sample extracts of (acid acetic in ethanol) were concentrated to one quarter of the original volume and precipitated the alkaloids by dropwise addition of concentrated NH_4OH until the pH is 10. Then they were collected by centrifugation. Each precipitate was washed with 1% NH_4OH and recentrifugated. After, they were collected, dried and stored at 4°C for further tests.

Antioxidant and antibacterial activities

The antioxidant and antibacterial activities of ethyl acetate fraction, butanolic fraction and total alkaloids of *Salvadora persica* stems and leaves were assessed.

(1) Antioxidant activity

Free radical scavenging activity

The procedure of Brand-Williams et al.[28] was adapted for evaluation of the free-radical scavenging capacity of sample extracts. Briefly, dried extracts were dissolved in methanol why obtained different concentration (1, 2.5, 5, 7.5, 10 mg/ml) of each one. The assay mixture contained in total volume of 1 ml, 500 µl of the extract, 125 µl prepared DPPH (1mM in methanol) and 375 µl of methanol. Ascorbic acid was used as a positive control. After 30 min incubation at 25°C, the decrease in absorbance was measured at λ=517 nm in JEN WAY 6405 UV/Vis spectrophotometer against blank of each concentration (extract plus methanol). The capacity to scavenge the DPPH radical was calculated as follows:

$$\textbf{Radical scavenging activity percent} = \frac{(A - B)}{B} \times 100$$

Where, A is the absorbance of the negative control (DPPH plus methanol) and B is the absorbance of the sample (DPPH, methanol plus sample). The correlation between each concentration and its percentage of scavenging was plotted, and the EC_{50} was calculated by interpolation. The activity was expressed as EC_{50} (the effective concentration of each extract that scavenges 50% of DPPH radicals).

Iron reducing power

The capacity of plant extracts to reduce Fe^{3+} was assessed by the method of Oyaizu[29].each dried extract were dissolved with methanol and different concentration (100, 250 and 750 µg/ml) were prepared. One milliliter of each one was mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. After that, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 3000g for 10 min. The upper layer frac-

tion (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride and thoroughly mixed. The absorbance was measured at 700 nm and ascorbic acid was used as a positive control. The EC50 value (µg/ml) is the extract concentration at which the absorbance was 0.5 for the reducing power and was obtained from the linear regression equation prepared from the concentrations of the extracts and the absorbance values. A higher absorbance indicates a higher reducing power.

(2) Antibacterial activity

Growth inhibition activities for sample extracts against (*Staphylococcus aureus* ATCC 25923, *Enterococcus feacalis* ATCC 29212, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Listeria monocytogenes* ATCC 19111) were tested using disc diffusion method as described by Berghe and Vlietinck[30]. The suspension of bacteria of about 1.5×10^6 CFU/ml "colony forming units per milliliter obtained following a 0.5 McFarland turbidity standard, which was standardized by adjusting the optical density to 0.1 at 600nm (JEN WAY 6405UV/Vis spectrophotometer)[31]. Inoculums' suspensions were diluted to 1/100 except the one of *Staphylococcus aureus* was diluted to 1/10[32]. They were used to inoculate by flooding the surface of Mueller-Hinton agar plates. Excess liquid was air-dried under a sterile hood. Dried extract were dissolved in DMSO at the concentration 5, 10 and 20 mg/ml. After, sterilized discs (Whatman n°1, 6 mm diameter) were impregnated with 5 µl of each extract (equivalent to 25, 50 and 100 µg/disc respectively) and placed on the agar surface. DMSO was used as negative control. The plates were left for 30mn at room temperature to allow the diffusion of extract, and then they were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone and presented in millimeter.

TABLE 1 : Field of different extracts from *Salvadora persica*

	Stems (%)	Leaves (%)
Methanol extract	7.08 ± 0.12	10.92 ± 0.33
Acetone extract	6.68 ± 0.1	10 ± 0.2
Ethyl acetate extract	0.2 ± 0.03	0.36 ± 0.16
Butanolic extract	0.32 ± 0.11	0.56 ± 0.11
Alkaloids extract	0.72 ± 0.24	1 ± 0.1

Statistical analysis

Assays were performed in triplicate for each sample. Results were expressed as mean values± standard deviation (SD). Correlation and regression analysis were carried out using the Excel program.

RESULTS

Extract yield

TABLE 1 showed the extraction yielding obtained for each extraction from *Salvadora persica* stems and leaves. The most yielding was obtained from different extract of leaves in comparison with stems. We observed that no significant difference between acetonic extract and methanolic extract. Alkaloids extract yield was more important (1 ± 0.1%) for leaves and (0.72 ± 0.24%) for stems followed by butanolic extract and ethyl acetate extract.

Determination of total phenolic, tannin and flavonoid content

The amount of total phenolic was higher in leaves 67.32 ± 0.17 mg PE/g DW than stems 58.63 ± 00 mg PE/g DW (TABLE 2). The same tendency was observed as in flavonoid content as in tannin content that leaves was 0.47 ± 0.00 mg RE/g DW and 39.31 ± 3.81 mg PE/g DW. But stems was 0.31 ± 0.01 mg RE/g DW plus 36.83 ± 2.76 mg PE/g DW.

Antioxidant activity

(1) Free radical scavenging activity

The stable DPPH radical is widely used to evaluate the free radical scavenging activity in many plant extracts. The results of DPPH (TABLE 3) test showed that Ethyl acetate extract (Leaves) was the most active with an IC_{50} value of 11.8 ± 0.07 µg/mlfollowed by Butanolic extract (Stems), Butanolic extract (Leaves), Alkaloids extract (Leaves), Ethyl acetate extract (Stems) and Alkaloids extract (Stems) with IC_{50} values

TABLE 2 : Ttotal phenolic, flavonoid and tannin of different extracts from *Salvadora persica*

	Stems	Leaves
Total phenolic (mg PE/g dw)	58.63 ± 00	67.32 ± 0.17
Total flavonoid (mg RE/g dw)	0.31 ± 0.01	0.47 ± 0.00
Total tannin (mg PE/g dw)	36.83 ± 2.76	39.31 ± 3.81

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TABLE 3 : Results of free radical scavenging activity and reducing power of different extract from *Salvadora persica*

Extract	IC_{50} (µg/ml) on DPPH	IC_{50} (µg/ml) on reducing power
Ethyl acetate extract (Stems)	7817 ± 63	20124 ± 670
Ethylacetate extract(Leaves)	11.8 ± 0.07	480 ± 6
Butanol extract (Stems)	14 ± 0.1	3290 ± 13
Butanol extract (Leaves)	257 ± 21	3660 ± 29
Alkaloids extract (Stems)	14891 ±103	29520 ± 798
Alkaloids extract (Leaves)	6233 ± 58	6680 ± 28
Ascorbic acid	10 ± 0.1	42 ± 8

of 14 ± 0.1, 257 ± 21, 6233 ± 58, 7817 ± 63 and 14891 ±103 µg/ml, respectively. These plant extracts showed lower radical scavenging activity compared to Ascorbic acid (IC_{50}, 10 ± 0.1 µg/ml).

(2) Iron reducing power

Iron reducing power of a compound may serve as indicator of its potential antioxidant activity. As shown in TABLE 3 the reducing power of Ethyl acetate extract (Leaves), expressed as IC_{50}, was higher than other extract plant (480 ± 6 µg/ml), followed by Butanolic extract (Stems), Butanolic extract (Leaves), Alkaloids extract (Leaves), Ethyl acetate extract (Stems) and Alkaloids extract (Stems) with IC_{50} values of 3290 ± 13, 3660 ± 29, 6680 ± 28, 20124 ± 670 and 29520 ± 798 µg/ml. Ascorbic acid was a higher reducing activity ($IC_{50,}$ 42 ± 8 µg/ml).

(3) Antibacterial activity

TABLE 4 showed the antibacterial activity of leaves and stems measured by the agar diffusion method against selected pathogenic bacteria. On the contrary of antioxidant activity who Ethyl acetate extract (Leaves) was a higher activity, Alkaloids extract (Stems) showed good inhibitory effects toward all bacteria as compared to other extract. The strongest activity of *Salvadora persica* extract was recorded also by them against *Bacillus cereus* ATCC 11778 (17mm at 20 mg/ml). Ethyl acetate extract (stems and leaves) revealed the lowest inhibitory effects.

DISCUSSION

In this study, extract yield, total phenolic, tannin and flavonoid content, antioxidant and antimicrobial activities of *Salvadora persica* stems and leaves were determined.

TABLE 4 : Antibacterial activity of each extracts from *Salvadora persica* (stems and leaves) at different concentration

Extract (mg/ml)	Inhibition zone (mm) against					
	Lm	Ef	Ec	Pa	Sa	Bc
Stems						
AE						
5	7	-	-	-	-	10
10	9	-	10	-	-	12
20	11	-	11	-	-	15
Bu						
5	9	6	6	-	7	6
10	10	7	8	-	9	8
20	11	10	10	9	11	10
Al						
5	10	10	-	-	-	10
10	12	12	8	7	7	14
20	13	13	9	10	12	17
Leaves						
AE						
5	-	-	-	-	-	9
10	-	7	10	-	-	12
20	10	11	11	-	10	14
Bu						
5	10	-	6	-	7	-
10	11	-	8	-	9	-
20	13	10	10	8	10	9
Al						
5	7	11	-	-	6	-
10	10	12	-	8	7	-
20	12	14	8	11	11	7

By comparing our results of extract yield with the bibliography, one found of stems aqueous methanolic extract in Souri et al.[33] is slightly different (7.4%) of our study (7.08%). In addition to the quantitative aspects, regardless of the extraction method applied it must take into account quality extract, in other words, the bio-activity of these active compounds. In Souri et al.[33] extraction lasted several days.

In our study, by (ULTRA TURRAX, IKA^{R} WERKE) extraction method, we have accelerated the process of extracting and minimized the time of contact with the extract solvent, while preserving the bio-activity of its constituents. Similarly, cold this temperature, extraction flow along the exhaustion of the solvent at

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reduced pressure, allows obtaining the maximum of compounds and preventing their denaturing, or likely change due to the temperatures used in other methods of extraction.

Results of extract Yield indicated that *Salvadora persica* was a higher yield of alkaloids than flavonoids. An indole alkaloid was reported in the leaves[34]. Whereas, the flavonoids Rutin and Quercetin were detected in the stems[35].

The determination of the total phenolic, flavonoid and tannin content of stems and leaves revealed that The levels of total polyphenol in stems (58.63 ± 00 mg PE/g DW) were superior to those reported by Alzoreky and Nakahara[36](1.6 ± 0.6 mg gallic acid equivalent/g Dw).

Concerning tannins, most authors reported their presence to insignificant concentration in *Salvadora persica* however our study proved the contrary. The lower flavonoid content observed in our results was in agreement with previous reports[37-39].

Because of the multiple ways in which an antioxidant can protect biological molecules against oxidative damage, we measured different reactions to assess antioxidant activity, so as to determine the true antioxidant potential of stems and leaves[40].

The antioxidant activity of each extract studied by us has not been reported before. Literature survey indicated antioxidant activity for stems aqueous methanolic extract using the ferrylmyoglobin /$ABTS^+$ assay[36].

Our study demonstrated the higher activity of leaves Ethyl acetate extract. The strong antioxidant property of this extract is associated to their phenols including falvonoids. This family from polyphenols have long been considered beneficial for health originally their good effects were thought to be due to their "antioxidative" effect and also their radical scavenging ability[41]. The scavenging activity of flavonoids depends to a high degree on their structures and physicochemical properties[42].

Results of antibacterial activity showed, that alkaloids extracts were more effective against bacteria, especially the one of stems. The extract of the stems is reported to possess antimicrobial activity[43-45]. When the stems is widely used as a tooth brush and to detoxify and strengthen the weakened gums; it is considered an efficient and inexpensive tool for oral hygiene[43].

Moreover, alkaloids have commonly been found to possess antimicrobial properties[46].

CONCLUSION

The results of the present study suggest that *Salvadora persica* stems and leaves contain a considerable amount of phenolic, tannin and alkaloid compounds, and had significant antioxidant and antimicrobial activity. More detailed studies on chemical composition of this plant, as well as other in vivo assays are essential to characterize them as biological antioxidants and antibiotic which are beyond the scope of this study.

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