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Research Article

Comparative Assessment of Antioxidant Activity and Phytochemical Analysis of Facultative Halophyte *Salvadora oleoides* Decne. and *Salvadora persica* L.

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Abstract

Background and Objective: Plants are important sources of biologically active natural products, which differ widely in terms of structure. Halophytes, distributed from coastal regions to inland deserts have traditionally been used for medicinal and nutritional purposes. Living in sub-optimal conditions, these plants synthesize stress associated bioactive molecules, which are still remain largely unexplored. The aim of the present study was to analyze antioxidant properties as well as phytochemical and physicochemical parameters from 2 species of *Salvadora*. **Materials and Methods:** The leaf, young stem (YS) and woody stem (WS) of *Salvadora oleoides* (*S. oleoides*) and *Salvadora persica* (*S. persica*) were collected and extracted individually by cold percolation method using different organic solvents like petroleum ether (PE), ethyl acetate (EA), acetone (AC), methanol (ME) and aqueous (AQ). Different solvent extracts of the both plants were subjected to antioxidant activity and quantitative phytochemical analysis. The qualitative phytochemical and physicochemical analyses were carried out from the crude powder. All the experiments were performed in triplicate and results were presented as mean \pm SEM. **Results:** Different solvent extracts of *S. persica* had higher phenolic content and antioxidant activity as compared to *S. oleoides*. **Conclusion:** This study will enable a better understanding as regards the significant correlation between phenolic content and antioxidant activity.

Key words: Facultative halophyte, antioxidant activity, phytochemical analysis, *Salvadora oleoides*, *Salvadora persica*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salvadora spp. has ability to grow in saline regions and arid to semiarid regions, therefore, there are possibilities for uses different parts in the medicinal purposes. The leaf of *S. oleoides* is used in open wound, blood purifiers and cooling agent. Stem is anthelmintic, diuretic property, fever, asthma, cough, leprosy, rheumatism¹. *Salvadora persica* stem is used in gastric troubles, vesicant and stimulant, while leaves are used in cough, piles, tumors, rheumatism and asthma². *Salvadora* species reported for various biological activities due to the presence of bioactive constituents viz., sitosterol, alkaloids, glucosides, rutin, terpenoids, phospholipids, dihydro-isocoumarin, resin, trimethyl amine, β -sitosterol, di-benzyl thiourea, rutin, thioglucoside, chlorine, potash and sulphur³⁻⁵.

As novel source of phenolic molecules, halophytes are known for their ability to withstand in harsh conditions and scavenge various toxic reactive oxygen species, since they harbor with a potent antioxidant system that includes polyphenolic compounds⁶⁻⁸. In recent year, different parts of halophyte species are being used all over the world^{9,10}. However, phytochemical analyses of these plants still need to be explored for medicinal purpose because of their capacity to accumulate and synthesize a polyphenolic compounds and ultimately have higher antioxidants, when the plant faces various biotic and abiotic stress, i.e., salinity¹¹. Hence to explore the halophytes for their antioxidant properties for human health, now a day's, people have more demanding on natural resources. It is speculated that, this is a first report on comparative study of antioxidant and its phytochemical properties of different solvent extracts of leaf, young stem and woody stem of *Salvadora oleoides* Decne. and *Salvadora persica* L. Therefore, the aim of the present study was the comparative assessment of antioxidant and phytochemical analysis of different solvent extracts of both *Salvadora* species using different *in vitro* assays.

MATERIALS AND METHODS

Plant collection: The leaf, young stem (YS), woody stem (WS) of *S. oleoides* and *S. persica* were collected in September, 2014 from Mudi, Surendranagar, Gujarat, India and in August, 2014 from Balachadi, Jamnagar, Gujarat, India, respectively, i.e., arid to semiarid and saline region of the Gujarat state. The plant parts were washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in air tight bottles.

Chemicals: Analytical grade of all chemicals and reagents were purchased: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ABTS (2, 2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid), 2,4,6-Tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu's reagent, sodium carbonate, aluminium chloride, potassium acetate, quercetin, glacial acetic acid, ammonium hydroxide, gallic acid, ascorbic acid, potassium persulfate, sodium acetate, sodium sulphate (Na_2SO_4), petroleum ether, ethyl acetate, acetone, methanol, chloroform, hydrochloric acid (HCl), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide reduced (NADH), nitro blue tetrazolium (NBT), ferric chloride (FeCl_3) from Sigma Chemical Co. (St Louis, MO, USA), MERCK Pvt. Ltd. (India), Hi-media (India) and Sisco Research Laboratories Pvt. Ltd. (India). Water was purified with a Milli-Q system (Millipore, Bedford, USA).

Plant extraction: The dried powder of the plant parts was extracted individually by cold percolation method^{12,13} using different organic solvents like petroleum ether (PE), ethyl acetate (EA), acetone (AC), methanol (ME) and aqueous (AQ). Ten grams of dried powder was taken in 150 mL petroleum ether in a conical flask, plugged with cotton wool and then kept on shaker at 120 rpm for 24 h. After 24 h, it was filtrated through 8 layers of muslin cloth, centrifuged at 5000 rpm in a centrifuge for 15 min and the supernatant was collected and the solvent was evaporated using a rotary vacuum evaporator (Equitron, India) to dryness. This dry powder was then taken individually in 150 mL of each solvent (ethyl acetate, acetone, methanol and water) and kept on a shaker at 120 rpm for 24 h. Then the procedure followed in each case was the same as above and the residues were weighed to obtain the extractive yield of all the extracts and were stored in air tight bottles at 4°C.

Antioxidant activity: The antioxidant activity of ethyl acetate, methanol and aqueous extracts of different parts were evaluated by DPPH free radical and superoxide anion radical, ABTS radical cation scavenging activity and ferric reducing antioxidant power (FRAP).

Determination of DPPH radical scavenging capacity: The DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging capacity of different solvent extracts was measured by using DPPH by the modified method^{14,15}. The reaction mixture (3.0 mL), consisted of 1.0 mL DPPH in methanol (0.3 mM), 1.0 mL methanol and 1.0 mL of different concentrations (upto 1000 $\mu\text{g mL}^{-1}$) of different solvent extracts diluted by methanol, was incubated for 10 min in dark, after which the

absorbance was measured at 517 nm using a digital spectrophotometer (Systronic, India), against a blank sample. Ascorbic acid (2-16 $\mu\text{g mL}^{-1}$) was used as positive control¹⁶. Percentage of inhibition was calculated using the following formula⁸:

$$\text{Inhibition (\%)} = 1 - \frac{A}{B} \times 100$$

Where:

B = Absorbance of blank (DPPH+methanol)

A = Absorbance of sample (DPPH, methanol+sample)

Determination of superoxide anion radical scavenging activity:

The superoxide anion radical scavenging activity of the different solvent extracts was measured by modified method^{17,18}. Superoxide radical was generated by oxidation of NADH and assayed by the reduction of NBT. The reaction mixture 3.0 mL consisted of 1.0 mL of the solvent extracts of difference concentration of extracts diluted by distilled water, 0.5 mL Tris-HCl buffer (16 mM, pH 8), 0.5 mL NBT (0.3 mM), 0.5 mL NADH (0.93 mM) and 0.5 mL PMS (0.12 mM). The superoxide radical generating reaction was started by the addition of PMS solution to the mixture. The reaction mixture was incubated at 25°C for 5 min and then the absorbance was measured at 560 nm using digital spectrophotometer (Systronic, India), against a blank sample. Gallic acid (50-225 $\mu\text{g mL}^{-1}$) was used as a positive control¹⁹. Percentage of inhibition was calculated as described above.

Determination of ABTS radical cation scavenging activity:

The ABTS (2, 2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid) radical cation scavenging activity of the different solvent extracts was determined by the modified method^{20,21}. ABTS radical cations were produced by reaction of ABTS (7 mM) with potassium persulfate (2.45 mM) and incubating the mixture at room temperature in the dark for 16 h. The working solution obtained was further diluted with methanol to give an absorbance of 0.85 ± 0.20 . One milliliter of different concentrations of solvent extracts diluted by methanol was added to 3.0 mL of ABTS working solution. The absorbance was measured at 734 nm using digital spectrophotometer (Systronic, India), against a blank sample. Ascorbic acid was used as a positive control²². Percentage of inhibition was calculated as described above.

Ferric reducing antioxidant power (FRAP): The reducing ability of different solvent extracts was determined by FRAP

assay^{23,24}. The FRAP assay was based on the ability of antioxidant to reduce Fe^{+3} - Fe^{+2} in the presence of TPTZ, forming an intense blue Fe^{+2} -TPTZ complex with an absorption maximum at 593 nm. This reaction was pH-dependent (optimum pH 3.6), 0.1 mL extract was added to 3.0 mL FRAP reagent [10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 1 part 20 mM FeCl_3] and the reaction mixture was incubated at 37°C for 10 min and then the absorbance was measured at 593 nm. FeSO_4 (100-1000 $\mu\text{M mL}^{-1}$) was used as a positive control. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as M FeSO_4 equivalents per gram of extracted compound²⁵.

Quantitative phytochemical analysis: Total phenol and flavonoid content were estimated in ethyl acetate, methanol and aqueous extracts of different parts of *S. oleoides* and *S. persica*. Total alkaloid content was estimated from the crude powder of different parts.

Determination of total phenol content: The amount of total phenol content of different solvent extracts was determined by Folin-Ciocalteu's reagent method^{26,27}. The extract (0.5 mL) and 0.1 mL of Folin-Ciocalteu's reagent (0.5 N) were mixed and the mixture was incubated at room temperature for 15 min. Then, 2.5 mL of sodium carbonate (2 M) solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm using a digital spectrophotometer (Systronic, India), against a blank sample. The calibration curve was made by preparing gallic acid (10-100 $\mu\text{g mL}^{-1}$) solution in distilled water. Total phenol content was expressed in terms of gallic acid equivalent (mg g^{-1} of extracted compound)²⁸.

Determination of flavonoid content: The amount of flavonoid content of different solvent extracts was determined by aluminium chloride colorimetric method^{29,30}. The reaction mixture (3.0 mL) consisted of 1.0 mL of sample (1 mg mL^{-1}), 1.0 mL methanol, 0.5 mL of aluminium chloride (1.2%) and 0.5 mL potassium acetate (120 mM) and was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm using a digital spectrophotometer (Systronic, India), against a blank sample. The calibration curve was made using quercetin (5-60 $\mu\text{g mL}^{-1}$) in methanol. The flavonoid content is expressed in terms of quercetin equivalent (mg g^{-1} of extracted compound)¹⁸.

Determination of total alkaloid content: One gram of dried powder was taken in 100 mL flask and 40 mL of 20% glacial acetic acid in methanol was added to it and was allowed to stand for 4 h at room temperature. This was filtered and the extract was concentrated in a water bath to one quarter of the original volume. Concentrated ammonium hydroxide (25%) was added drop wise to the extract until the precipitation was complete. Then it was taken in a separating funnel and an equal volume of chloroform was added. It was washed with distilled water 3 times to make it pH neutral. Sodium sulphate (Na_2SO_4) was added to remove moisture. It was filtered and dried. Crude alkaloid content was collected and weighed. Crude alkaloid content was expressed in mg g^{-1} of dried powder^{31,32}.

Qualitative phytochemical analysis: The crude powder of *S. oleoides* and *S. persica* was subjected to qualitative phytochemical analysis³¹.

Physiochemical analysis-Determination of total ash: Two grams of dry powder was taken in a silica crucible and heated gradually increasing the heat to 500°C until it was white, indicating the absence of carbon. Ash was cooled in a desiccator and weighed without delay. Total ash value was calculated was mg g^{-1} of air dried material^{33,34}.

Determination of water soluble ash: To the crucible containing the total ash, 25 mL of water was added and boiled for 5 min. The insoluble matter was collected on an ash less filter paper. It was washed with hot water and heated in a crucible for 15 min. weight of insoluble matter was subtracted from the weight of total ash. The content of water soluble ash was calculated in mg g^{-1} of air dried material^{33,35}.

Determination of acid insoluble ash: Twenty five milliliter of hydrochloric acid (70 g L^{-1}) was added to the crucible containing total ash. It was covered with a watch-glass and heated gently for 5 min to boil. The watch-glass was rinsed with 5 mL of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and it was washed with hot water until the filter was neutral. The filter paper containing the insoluble matter was transferred to the original crucible; it was dried on a hot plate and heated till constant weight was obtained. The residue was allowed to cool in desiccators for 30 min and then weighted without delay. Acid insoluble ash was calculated in mg g^{-1} of air dried material^{33,36}.

Statistical analysis: All the experiments were performed in triplicate and results are presented as mean \pm SEM (Standard Error of Mean).

RESULTS AND DISCUSSION

Based on the results, it is significantly observed that the higher amount of the phenolic content in the extracts shown remarkable antioxidant activity in a positive manner. Phenolic compounds is a major class of the natural compounds those are reported for the potent antioxidant activity may be because of the hydrogen donating capacities of the components present in the extracts.

Antioxidant activity: The plants have effective capacity to scavenge free radicals and represent a source of multifunctional properties. Medicinal plants are rich in secondary metabolites that exhibit a remarkable diversity of both chemical structures and biological activities and are promising source of lead compounds for new drugs targeting neurodegenerative diseases^{37,38}. Antioxidant methods and modifications have been proposed to evaluate antioxidant characteristics and to explain how antioxidant functions. It is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals. Therefore, in the present study, DPPH free radical scavenging activity, Superoxide anion radical scavenging activity, ABTS radical cation scavenging activity and ferric reducing antioxidant power were performed. The IC_{50} values are negatively correlated to the antioxidant activity as it expresses the amount of antioxidant needed to decrease its radical concentration by 50%; the lower IC_{50} value represents the higher antioxidant activity of the tested sample³⁹.

The DPPH assay is a general test used to evaluate the antioxidant capacity of plant extracts. The DPPH radical is commonly used for fast evaluation of the antioxidant property of a given compound. Being a stable free radical, DPPH is frequently used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbed at 517 nm but upon reduction with an antioxidant, its absorption decreases due to the formation of its non-radical form DPPH^{40,41}. Thus, the radical scavenging activity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution. The colour change from purple to yellow after reduction can be quantified by decrease of absorbance at wave length 517 nm.

Table 1: IC₅₀ values of DPPH free radical, superoxide anion radical and ABTS radical cation scavenging activity of different solvent extracts of leaf and stem of *Salvadora* species

Plant names	Parts used	IC ₅₀ values (µg mL ⁻¹)								
		DPPH			SO			ABTS		
		EA	ME	AQ	EA	ME	AQ	EA	ME	AQ
<i>Salvadora oleoides</i>	Leaf	>1000	427.5	>1000	>1000	>1000	>1000	440	360	576
	YS	>1000	736	>1000	>1000	>1000	>1000	232	184	622.5
	WS	>1000	>1000	616	>1000	>1000	>1000	316	212	280
<i>Salvadora persica</i>	Leaf	>1000	750	>1000	>1000	>1000	>1000	140	320	320
	YS	>1000	>1000	>1000	>1000	>1000	>1000	466	280	360
	WS	248	824	>1000	>1000	>1000	>1000	120.5	216	368
Standard		Ascorbic acid	11.4		Gallic acid	185		Ascorbic acid	6.4	

EA: Ethyl acetate, ME: Methanol, AQ: Aqueous, DPPH: DPPH (2,2-Diphenyl-1-picryl hydrazyl) free radical scavenging activity, SO: Superoxide anion radical scavenging activity, ABTS: ABTS (2,2'-Azino-bis-(3-ethyl)benzothiazoline)-6-sulfonic acid diammonium salt) radical cation scavenging activity

It is usually expressed as IC₅₀ value, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50% lower IC₅₀ value indicates high antioxidant activity^{42,43}. The IC₅₀ values of DPPH free radical scavenging activity of different solvent extracts of leaf, YS and WS of *S. oleoides* and *S. persica* are shown in Table 1. Out of 18 extracts investigated, 12 extracts showed IC₅₀ value more than 1000 µg mL⁻¹, while remaining 6 extracts showed varied levels of DPPH free radical scavenging activity, IC₅₀ value ranged from 248-824 µg mL⁻¹. Ascorbic acid was used as a standard and its IC₅₀ value was 11.4 µg mL⁻¹. Amongst all the ethyl acetate and aqueous extracts, *S. persica* WS and *S. oleoides* WS, the IC₅₀ value was 248 and 616 µg mL⁻¹, respectively, while remaining all extracts showed IC₅₀ value more than 1000 µg mL⁻¹. Methanol extracts showed better DPPH radical scavenging activity as compare to other extracts and *S. persica* showed better DPPH radical scavenging activity as compare to *S. oleoides*.

Superoxide and hydroxyl radical are the two most dangerous free radicals constantly produced in living organisms. Superoxide is considered as major biological source of ROS and they are harmful because they may oxidize and reduce other compounds and potentially form more reactive species via secondary reactions. Therefore, superoxide scavenging capacity in the human body is very important as the first line of defense against oxidative stress^{38,44}. The IC₅₀ values of super oxide anion radical scavenging activity of different solvent extracts of leaf, YS and WS of *S. oleoides* and *S. persica* are shown in Table 1. Out of 18 extracts investigated, all 18 extracts showed IC₅₀ value more than 1000 µg mL⁻¹. Gallic acid was used as a standard and its IC₅₀ value was 185 µg mL⁻¹. This result indicates poor ability of the extracts in the quenching of superoxide anion radical.

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of

chain breaking antioxidants. ABTS assay is better to assess the antiradical capacity of both hydrophilic and lipophilic antioxidant because it can be used in both organic and aqueous solvent system as compared to other antioxidant assay^{25,45}. This method is based on the ability of antioxidant to reduce the ABTS radical cation. The IC₅₀ values ABTS radical cation scavenging activity of different solvent extracts of leaf, YS and WS of *S. oleoides* and *S. persica* are shown in Table 1. All the 18 extracts investigated showed varied levels of ABTS radical cation radical scavenging activity, IC₅₀ value ranged from 120.5-622.5 µg mL⁻¹. Ascorbic acid was used as a standard and its IC₅₀ value was 6.4 µg mL⁻¹. Amongst all the different solvent extracts, ethyl acetate extracts of *S. persica* WS (120.5 µg mL⁻¹) and leaf (140 µg mL⁻¹) showed best ABTS radical cation scavenging activity. Ethyl acetate and methanol extracts showed better ABTS radical cation scavenging activity as compare to aqueous extracts and *S. persica* showed better ABTS radical cation scavenging activity as compare to *S. oleoides*.

FRAP assays is based on the ability of an antioxidant to reduce Fe⁺³-Fe⁺² in the presence of TPTZ, forming an intense blue Fe⁺²-TPTZ complex with an absorbance decrease is proportional to the antioxidant content^{46,47}. Ferric reducing antioxidant power of different solvent extracts of leaf, YS and WS of *S. oleoides* and *S. persica* is shown in Table 2. Among the different solvent extracts of both plants, the highest FRAP activity was in methanol extract of *S. persica* leaf, followed by methanol and aqueous extracts of *S. persica* WS. Polar solvent extracts showing better FRAP activity as compare to Ethyl acetate extracts.

Quantitative phytochemical analysis: Phytochemicals are produced by specific biochemical pathways, which occur inside the plant cells. A number of phytochemicals isolated

Table 2: Total phenol content, flavonoid content and ferric reducing antioxidant power of different solvent extracts of different parts of *Salvadora* species

Plant names	Parts used	Extracts	TPC (mg g ⁻¹)*	FC (mg g ⁻¹)*	FRAP (M g ⁻¹)*
<i>Salvadora oleoides</i>	Leaf	EA	127.66±5.33	47.17±0.74	19.95±0.19
		ME	253.10±9.83	43.65±0.10	94.19±0.81
		AQ	222.89±10.00	17.07±0.22	72.85±0.89
	YS	EA	114.96±2.36	16.16±1.82	14.80±0.05
		ME	235.09±3.44	8.60±0.02	82.00±0.73
		AQ	215.52±3.26	1.22±0.06	51.09±1.64
	WS	EA	178.26±1.88	13.59±1.51	30.85±0.03
		ME	241.15±1.42	25.60±0.78	92.83±1.48
		AQ	209.79±0.65	0.46±0.06	53.36±0.44
<i>Salvadora persica</i>	Leaf	EA	199.30±2.34	44.47±1.21	63.47±2.32
		ME	252.77±1.13	57.94±0.41	151.04±19.40
		AQ	212.32±7.50	39.15±0.39	94.11±1.82
	YS	EA	269.97±1.13	42.22±0.12	67.44±2.45
		ME	237.79±1.21	43.11±0.54	299.95±9.30
		AQ	238.12±1.15	23.64±0.30	106.93±1.88
	WS	EA	28.66±0.59	30.01±0.39	1.01±0.12
		ME	222.23±4.12	5.08±0.29	54.67±1.42
		AQ	182.52±1.85	0.00±0.00	35.68±0.49

*Values are expressed in mean±standard error of the mean (n = 3), TPC: Total phenol content, FC: Flavonoid content, FRAP: Ferric reducing antioxidant power, YS: Young stem, WS: Woody stem, EA: Ethyl acetate, ME: Methanol, AQ: Aqueous

Table 3: Total alkaloid content from crude powder of *S. oleoides* and *S. persica*

Plants	Parts	Total alkaloid content (mg g ⁻¹)
<i>Salvadora oleoides</i>	Leaf	0.61
	YS	0.19
	WS	0.20
<i>Salvadora persica</i>	Leaf	0.49
	YS	0.96
	WS	0.31

YS: Young stem, WS: Woody stem

from plant material are used in the pharmaceutical drug industry today. The phytochemicals under investigation include secondary metabolites, which are synthesized for plant defense and adaption to environmental stress³⁸. In the present study, different solvent extracts of different parts of *S. oleoides* and *S. persica* were evaluated for to estimate their total phenol content. The total phenol content of different solvent extracts of different parts of *S. oleoides* and *S. persica* are shown in Table 2. All the different solvent extracts of the different parts of the *S. persica* showed higher amount of total phenol content than *S. oleoides*. Amongst the different solvent used, polar solvents i.e., ME and AQ had more total phenol content as compare to non-polar solvent i.e., EA. The highest total phenol content was in ME of *S. persica* leaf, while lowest amount of total phenol content was in EA of *S. oleoides* leaf, YS and WS. According to these results, there is good relationship between total phenols and antioxidant activity. Overall, the antioxidant activities of the extracts were highly correlated with their total phenolic contents and these results are similar to those of previous findings⁴⁸⁻⁵⁰. In the present study, different solvent extracts of different parts of *S. oleoides* and *S. persica* were evaluated for to estimate their

flavonoid content. The flavonoid content of different solvent extracts of different parts of *S. oleoides* and *S. persica* are shown in Table 2. All the different solvent extracts of the different parts of the *S. oleoides* showed higher amount of flavonoid content than *S. persica* except leaf. Amongst the different solvent used, non-polar solvent i.e., EA had more flavonoid content as compare to polar solvents i.e., ME and AQ. The highest flavonoid content was in EA of *S. persica* YS followed by WS, while no flavonoid content was in AQ of all the different parts of both plant. The total alkaloid content of crude powder of *S. oleoides* and *S. persica* is shown in Table 3. Amongst all the different parts of both plant, the highest total alkaloid content was in *S. persica* YS followed by *S. oleoides* leaf (Table 3).

Qualitative phytochemical analysis: The result of qualitative phytochemical analysis of the crude power of *S. oleoides* and *S. persica* are shown in Table 4. Steroids is present in high amount in leaf and YS of *S. oleoides* and *S. persica*. Cardiac glycosides is present in high amount in YS of *S. oleoides* and leaf and YS of *S. persica*. Flavonoids and triterpenes are present in high amount in *S. oleoides* WS and *S. persica* WS respectively. Triterpenes is present in moderate amount in YS and WS of *S. oleoides*.

Physiological parameters: The physiological evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs⁵¹. Maximum amount of total ash and acid soluble ash were in *S. oleoides* leaf, while water soluble ash was in *S. persica* leaf (Table 5).

Table 4: Qualitative phytochemical analysis of *S. oleoides* and *S. persica*

Phytochemicals	<i>Salvadora oleoides</i>			<i>Salvadora persica</i>		
	Leaf	YS	WS	Leaf	YS	WS
Flavonoid	-	-	+++	-	+	+
Tannins	-	-	-	+	-	-
Phylobatanins	-	-	-	-	-	-
Saponins	+	+	+	-	-	+
Steroids	+++	+++	+	+++	+++	+
Cardiac glycosides	+	+++	-	+++	+++	-
Triterpenes	+	++	++	-	-	+++
Alkaloids						
Wagner's	-	-	-	-	-	-
Mayer's	++	+	-	-	-	+

YS: Young stem, WS: Woody stem, -: No presence, +: Less presence, ++: Moderate presence, +++: High presence

Table 5: Physicochemical parameters of *S. oleoides* and *S. persica*

Parameters	<i>Salvadora oleoides</i>			<i>Salvadora persica</i>		
	Leaf	YS	WS	Leaf	YS	WS
Total ash*	0.71	0.31	0.21	0.58	0.29	0.18
Water soluble ash*	0.12	0.08	0.08	0.22	0.16	0.16
Acid insoluble ash*	0.64	0.01	0.02	0.08	0.08	0.02

*[% Value (w/w)], YS: Young stem, WS: Woody stems

CONCLUSION

This study reports for the first time comparative assessment of these two *Salvadora* species for antioxidant property and their phytochemical analysis. Of the two species studied, *S. persica* exhibited high phenolic levels associated to potent antioxidant efficacy. This study reinforces the notion that phenolic compounds largely contribute to antioxidant activity. Obtained results pointed out a significance of further studies in phytochemistry and bioactivity of *S. persica* from arid, semiarid and saline habitats.

SIGNIFICANCE STATEMENTS

The present study reported for the 1st time on comparative study of antioxidant and its phytochemical profile of leaf, young stem and woody stem of *Salvadora oleoides* Decne. and *Salvadora persica* L. This study discovers the possible belief that phenolic compounds largely contribute to antioxidant activity. This study will help the researcher to uncover the positive relation of secondary metabolites and antioxidant activity of *S. persica* from arid, semiarid and saline habitats, since increased biosynthesis of secondary metabolites and related antioxidant activity seem to be induced under environmental stresses conditions.

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