

Phytochemical screening, total phenolic and flavonoid contents and antioxidant activity of *Anabasis setifera* and *Salsola tomentosa* extracted with different extraction methods and solvents

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Abstract In this study, the tests of phytochemical screening included phenols, tannins, flavonoids and saponins. Total phenolic was estimated by the Foline–Ciocalteu method, total flavonoid content was determined by a colorimetric method. Extracts were also monitored on their antioxidant ability by DPPH and β -carotene bleaching methods. The phytochemical screening of *A. setifera* and *S. tomentosa* dry aerial parts revealed the presence of phenolic components (tannins, flavonoids and total phenols), and saponins. Total phenolic and flavonoid were high with methanol extraction whereas acetone showed poor flavonoid and polyphenol content in both Soxhlet apparatus and maceration methods. Furthermore all the extracts have good antioxidant activities. This work will be helpful to explore the biochemical profile and active compound identification in the field of pharmaceutical research.

Keywords *Anabasis setifera* · *Salsola tomentosa* · Medicinal plant · Phytochemicals · Antioxidant activity

Introduction

Phytochemicals (such as terpenes, flavonoids, saponins, tannins, amino acids, fatty acids, sterols and alkaloids) provide

definite physiological action on the human body (Samejo et al. 2013). Some classes of these compounds are responsible for the biological activity of some plants and are bioactive in humans and animals. They are involved in a number of physiological functions in plants, including specialized plant growth, development and defense mechanisms (Rusak et al. 2008). These compounds have diverse chemical behavior, including different polarity. Thus, solubility in a particular solvent is specific for each compound, which explains the lack of a universal extraction procedure (Bussmann et al. 2011).

There are several methods established for the extraction of secondary metabolites from plant materials. Those methods vary in solvents and conditions used. The extraction method is essential for the accurate quantification of antioxidant content and capacity. This fact makes it hard to compare data from literature reports, due to the reason mentioned earlier (Bussmann et al. 2011; Rusak et al. 2008; Abeyasinghe et al. 2007; Jirovetz et al. 2006).

Chenopodiaceae is one of the most important families in such inhospitable places as deserts, semi-deserts, and salt marshes. Chenopods are often important sources of forage for grazing livestock. Some of them also provide a useful source of fuel, while others have been used as a commercial source of potash or alkali. Many members of this family are succulent and late flowering and fruiting, which has historically made collections difficult to identify, with many specimens lacking the necessary characters for species identification (Costa et al. 2015). *Anabasis setifera* and *Salsola tomentosa* belong to the *Chenopodiaceae* family. These plants have been used as medicinal herbs in Iran. The present study was made to identify the diversity of *A. setifera* and *S. tomentosa* in secondary metabolites by using different extracts along with antioxidant activity.

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Materials and methods

Collection of *A. setifera* and *S. tomentosa*

The aerial parts of plants were collected from growing plants sites in Qum province from Iran during the October. To be more exact about sampling, three samples were collected from each shrub and they were mixed then samples were labeled and locations were recorded using a Global Positioning System (GPS, Vista Garmin) receiver (Location: 39 S 494465 E, 3,873,592 N). After sampling, drying was done in the shade and the room temperature for 20 days.

Preparation of the extracts

For preparation of aqueous extracts, 50 g powdered of the areal parts of plants were extracted separately with double distilled water, acetone and methanol for 72 h. The extract was filtered (with Whatman no. 1 filter paper). The filtrate was used for phytochemical test.

In order to extract for phytochemical test and total phenolic and flavonoid content by maceration method, the areal parts of *A. setifera* and *S. tomentosa* were powdered and extracted for 24 h with 20 ml of solvents (acetone or methanol 96.6 °) at room temperature, followed by rapid paper filtration through Whatman No 0.45 mm filter paper. The resulting solutions were evaporated under vacuum at 60 °C by Buchi Rotavapor R-200 to dryness. The residues were then dissolved in 3 ml of solvents (acetone or methanol).

In order to extract for total phenolic and flavonoid content by a soxhlet apparatus, powdered shoots (30 g) were extracted in a soxhlet apparatus using two solvents (acetone or methanol 96.6 °). Afterwards, the extracts were filtered, and solvent was evaporated under reduced pressure using rotary vacuum evaporator. At last, extracts were freeze-dried and the residue was reconstituted in 3 ml of solvent (acetone or methanol) before testing (Benhammou et al. 2009).

Test for class of phytochemicals

The extracts were analyzed for the presence of phenolic compounds, tannins, terpenoids, saponins, and flavonoids (Oueslati et al. 2012; Samejo et al. 2013).

About 0.5 g of plant sample was boiled in 10 ml distilled water in test tube and then filtered. A few drops 0.1 % of FeCl₃ solution were added to the filtrate. Blue–black precipitate indicated the presence of phenols and tannins.

Terpenoids were tested by using 500 µL of extract mixed with 200 µL of chloroform in a test tube. Concentrated sulfuric acid (300 µL) was added to the mixture carefully and observed interface with a reddish brown coloration.

Saponins in the extracts were tested by adding 2 mL of extract in a test tube and were shaken vigorously to obtain a

stable persistent froth. Mixing of two drops of olive oil in the froth allowed for the formation of an emulsion, which indicated the presence of saponins.

Yellow color appearance when the extract (1 mL) was added with three drops of 1 % ammonium solution, which indicates the presence of flavonoids.

Total phenolic and flavonoid content

The total phenolic contents were determined with Folin–Ciocalteu method. Briefly, 0.1 ml of each phenolic extract was mixed with 2 ml of 7.5 % sodium carbonate and then the mixture was allowed to stand at room temperature for 5 min. After addition of 100 mL of Foline–Ciocalteu reagent (1 N), the mixture was left in the dark room for 30 min at room temperature. The absorbance was measured at 750 nm using a spectrophotometer. The analysis was performed in triplicate and the concentration of phenolic compounds was expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g) (John et al. 2015).

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Vermerris and Nicholson 2006; El–Haci et al. 2013). Each plant extract solution (500 µL) or standard solution of catechin was added to 10 mL volumetric flask containing 2 mL of double distilled water. Then 150 µL NaNO₂ (15 %) was added to the flask and after 6 min, 150 µL AlCl₃ (10 %) was also added. At 6th min, 2 mL NaOH (4 %) was added and the total volume was made up to 5 mL with double distilled water. The solution was mixed completely and the absorbance level was measured versus prepared reagent blank at 510 nm. Total flavonoid content was expressed as catechin equivalent per gram of dry extract (mg CEQ/g).

DPPH radical-scavenging assay

The antioxidant capacity of the extracts was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by Alothman et al. (2009). An aliquot (10 µl) of extract was mixed with 90 µl of distilled water and 3.9 ml of 25 mM DPPH[•] methanolic solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured later, at 515 nm, against a blank of methanol without DPPH[•]. Results were expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation:

%inhibition of DPPH

$$= \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100$$

Table 1 Class of phytochemicals in *A. setifera* and *S. tomentosa*

	<i>Anabasis setifera</i>			<i>Salaola tomentosa</i>		
	water	acetone	methanol	water	acetone	methanol
tannins	+	-	+	+	-	+
phenols	+	-	+	+	-	+
terpenoids	+	+	+	+	-	-
saponins	+	+	-	+	+	-
flavonoids	+	-	+	+	-	+

+: presence of phytochemicals; -: Absent of the phytochemicals

Where Abs control is the absorbance of DPPH[•] solution without extracts.

$$\text{Bleaching inhibition(\%)} = \left(\frac{\beta\text{-carotene content after 2 h of assay}}{\text{initial}\beta\text{-carotene content}} \right) \times 100$$

Statistical analysis

All the experiments were carried out in triplicates. Tests of significant differences between means were determined by Duncan's multiple range tests at a significance level of 0.05 and values were expressed as means \pm SD (standard deviations) using SPSS program (Version 21).

Results

The plant extracts were tested for its various class of phytochemicals. The results are presented in Table 1. In both *A. setifera* and *S. tomentosa* the tannins, phenols and flavonoids were observed with methanol and water. The terpenoids were observed with all solvent in *A. setifera*; whereas it was observed with water in *S. tomentosa*. Also, in both of the plants, saponins were observed with water and acetone. Generally, all class of phytochemical were observed with water in both *A. setifera* and *S. tomentosa*.

β -carotene bleaching assay

The β -carotene bleaching assay was done according to the method of Zarai et al. (2013) with some modifications. A stock solution was prepared as follows: 0.5 mg β -carotene, 25 μ l of linoleic acid and 200 μ l of tween 80 were dissolved in 1 ml of chloroform (HPLC grade). Chloroform was completely evaporated at 40 °C, then, 100 ml of distilled water was added with vigorous shaking. The reaction medium contained 500 μ l of each extract solution and 2.5 ml of the freshly prepared emulsion. All the test tubes were immediately placed in a water bath at 50 °C for 2 h. The absorbance was measured at 470 nm before and after heat treatment. β -Carotene bleaching inhibition was estimated using the following equation:

The total phenolics and flavonoids contents were measured with different methods and solvents are presented in Table 2. Results revealed that in all the extracts the amount of flavonoids was less than 5 mg CEQ/g. the highest level of this compound was 4.56 and 2.98 mg CEQ/g for extraction with maceration method and methanol solvent (in *S. tomentosa* and *A. setifera*, respectively). Furthermore, the highest level of total phenolic content were measured with maceration method and methanol solvent (31.73 and 23.05 mg GAE/g in *S. tomentosa* and *A. setifera*, respectively). Also, the amount of phenolics and flavonoid content measured with soxhlet apparatus method and methanol solvent were more that acetone, which suggests that the plants are rich in total phenolics and flavonoids.

DPPH and β -carotene radical scavenging activities of solvent extracts are shown in Table 3. Methanolic extracts of *A. setifera* (40.58) and *S. tomentosa* (36.29) extracted with maceration method showed significant antioxidant activities. Nevertheless the highest β -carotene radical scavenging activity in *A. setifera* was extracted with methanol and soxhlet apparatus (32.19 %) and the highest percentage

Table 2 Effect of different extraction methods and solvents on total phenolic and flavonoid content of *A. setifera* and *S. tomentosa*

	Total phenolic content (mg GAE/g) ^a				Total flavonoid content (mg CEQ/g) ^{ab}			
	maceration		soxhlet apparatus		maceration		soxhlet apparatus	
	acetone	methanol	acetone	methanol	acetone	methanol	acetone	methanol
<i>A. setifera</i>	7.25 \pm 0.01	23.05 \pm 0.07	20.18 \pm 0.02	20.68 \pm 0.02	0.81 \pm 0.01	2.98 \pm 0.03	2.61 \pm 0.02	2.72 \pm 0.01
<i>S. tomentosa</i>	20.37 \pm 0.01	31.73 \pm 0.09	13.97 \pm 0.01	25.26 \pm 02	2.08 \pm 01	4.56 \pm 02	0.80 \pm 0.01	2.40 \pm 0.00

^a Mean values ($n = 3$) with significant difference at $P < 0.05$

Table 3 Antioxidants activity of *A. setifera* and *S. tomentosa* plant extracts

	%DPPH radical scavenging activity ^{a,b}				%β-carotene bleaching ^{a,b}			
	maceration		soxhlet apparatus		maceration		soxhlet apparatus	
	acetone	methanol	acetone	methanol	acetone	methanol	acetone	methanol
<i>A. setifera</i>	28.62 ± 0.02	40.58 ± 0.03	16.39 ± 0.01	36.47 ± 0.06	21.08 ± 0.02	28.18 ± 0.04	14.45 ± 0.03	32.19 ± 0.02
<i>S. tomentosa</i>	24.87 ± 0.06	36.29 ± 0.05	18.70 ± 0.03	30.41 ± 0.03	24.31 ± 0.02	31.81 ± 0.01	16.13 ± 0.02	28.21 ± 0.01

^a Mean values ($n = 3$) with significant difference at $P < 0.05$

^b Percentage of inhibition

of it was extracted with methanol and maceration method (31.81 %) in *S. tomentosa*.

Discussion

The results of preliminary phytochemical screening suggest that the extracts of both *S. tomentosa* and *A. setifera* are good sources of beneficial class of phytochemical such as tannins, phenols, terpenoids, saponins and flavonoids. These phytoconstituents play a significant role in the medicinal properties of plants (Unekwu et al. 2014).

Saponins for example comprise a large family of structurally related compounds containing a steroid or triterpenoid aglycone. They are reported to have a wide range of beneficial pharmacological properties, such as anti-inflammatory and anti-diabetic effects (Thoppil and Bishayee 2011). Thus, these plants can be used in the management of diabetes and inflammation related diseases.

Terpenoids have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anti-cancer effects among others (Thoppil and Bishayee 2011).

Phenolic components are antioxidants, and exhibit a wide range spectrum of medicinal properties such as anti-cancer, anti-inflammatory and diabetic effects (Wang et al. 2005). Thus the extracts of the *A. setifera* and *S. tomentosa* may be good alternatives for the treatment of diseases associated with excessive free radical generation and damage.

Flavonoids are one of the most diverse groups of natural components that have been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, anti-allergenic, antiviral and anti-inflammatory effects (Yesilada et al. 2000).

The phenolics and flavonoids content of the *A. setifera* and *S. tomentosa* were determined by soxhlet apparatus and maceration method. Phenols are among the non-enzymatic compounds obtained from natural sources, which have received high attention due to their proven antioxidant capabilities. Although phenolic compounds have been related to antioxidant activity, some studies have emphasized specific classes

such as flavonoids and tannins (Sang et al. 2002). Similar to this study, according to Upadhyay et al. (2013) the total flavonoids were highly extracted by methanol whereas total phenolic compounds with acetone. It is due to solubility of the flavonoids were strongly affected by the nature of the solvent and the flavonoid structure (Valente et al. 2010). On the other hand, the bio-availability of the compounds plays a vital role in the extraction and its dissolving potential against various solvents. Hence, some other compounds like terpenoids dissolved highly with the methanol and acetone resulting in the variation of the extracts.

In the DPPH and β-carotene radical scavenging assay, all solvents and extraction methods exhibited different degrees of antioxidant activity as shown in Table 3. The methanol extracts from *A. setifera* and *S. tomentosa* had the highest antioxidant activity and total phenolics content. A positive correlation was observed between percentage antioxidant and total phenolic, which increased with the higher level of samples. These correlations confirm that the phenolic compounds are the main micro constituents contributing to the antioxidant activities of these plants. The present study confirms that various solvent extracts from *A. setifera* and *S. tomentosa* contain significant source of phenolic and flavonoid antioxidants that may have therapeutic potential.

Conclusion

The present study confirms the various class of phytochemicals of *A. setifera* and *S. tomentosa* and their total phenolic and flavonoid content. All the extracts exhibited antioxidant activity. It should be also noted that polyphenol contents were positively and statistically significantly correlated with the antioxidant activity of the studied extracts. This report indicates that the extracts of these plants could be used as natural antioxidant in both food preservation and human health.

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Compliance with ethical standards**Ethical Statement** N/A**Conflict of Interest** The authors have declared that no conflict of interest.**References**

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