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ANTIOXIDANT ACTIVITY OF *SENECIO CHRYSANTHEMOIDES* EXTRACTS

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INTRODUCTION

The genus *Senecio* (Asteraceae) is one of the richest in species among the Angiosperms, with more than 1500 species distributed worldwide in cold and tropical regions.[1] Many species of the genus *Senecio* have reportedly been used by Andean people in Chile as traditional remedies for mountain sickness. Several of these species are known by the vernacular name "*chachakoma*" but Villagran *et al*., pointed out that given the same name to widely different species of the genus *Senecio* is basically wrong and misleading.[2] In traditional medicine, the use of *Senecio* species for treatment of asthma, coughs, bronchitis, eczema and wound healing have been reported.[3-5] Previous works on the chemical composition of the essential oils of some *Senecio* included *Senecio trapezuntinus* Boiss., *Senecio platyphyllus* DC. var. *platyphyllus, S. vernalis* Waldst. & Kit., *S. glaucus* subsp. *coronopifloius, S. leucostachys* Baker., *Senecio squalidus* L., *Senecio aegyptius* var. *discoideus* Boiss., *Senecio graveolens* Wedd., *Senecio farfarifolius* Boiss., *Senecio nutans* Sch.-Bip., and *Senecio longipenicillatus* Sch.-Bip.[6] *S. chrysanthemoides* DC (Asteraceae) is a deciduous shrub found in north-west Himalayas (India).[7] The traditional claim associated with *Senecio* species and lack of scientific studies regarding antioxidant potential of *S. chrysanthemoides* prompted us to perform this study. The objective of the present study was to evaluate antioxidant potential of *S. chrysanthemoides* extracts. The present paper for the first time reports antioxidant activity of *S. chrysanthemoides* collected from north west Himalayas, India.

MATERIAL AND METHODS

Plants Materials

Whole plants of *Senecio chrysanthemoides* were collected from the Tungnath (Chopta), Rudraprayag, Uttrakhand India in Sep-October 2015. The plant was identified from Department of Botany, HNB Garhwal University Srinagar Uttrakhand. A Voucher Specimen (**GUH-3354**) was deposited in the Department of Botany.

Chemicals

Sodium carbonate, gallic acid, Folin-Ciocalteu reagent, rutin, ammonium molybdate, sodium phosphate, ferric chloride, potassium ferricyanide, trichloroacetic acid, 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), potassium persulfate, 2,2′-azinobis-(3-ethylbenzothiozoline-6-sulfonic acid)-diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferrozine, ferrous chloride, ethylene diaminetetracetic acid (EDTA) disodium salt, ascorbic acid; were obtained from Himedia chemicals and Sigma. All other reagents used were of analytical grade.

Plant material and extraction procedure

S. chrysanthemoides was collected (May-June) from north-west Himalayas (Uttarakhand, India) and identified by Department of Botany, H. N. B Garhwal University (A Central University), Uttarakhand, India. Freshly collected stem was shade dried and cut into small pieces. The plant material (5 kg) was extracted in Soxhlet extractor with petroleum ether, ethyl acetate and methanol. The extracts were concentrated by rotary vacuum evaporator (40°C) and air dried to afford crude extracts. The yield of ethyl acetate and methanolic extracts was 85 g and 200 g respectively. The crude extracts were used for evaluation of antioxidant activity.

Determination of total phenolic content

The total phenolic content (TPC) was determined according to reported method.[8] The reaction mixture consisted of 0.5 ml extract, 2.5 ml of the Folin-Ciocalteu's reagent (10% v/v) and 2.0 ml of saturated sodium carbonate solution. The resulting mixture was vortexed for 15 sec and incubated (40°C, 30 min) for color development. The absorbance of total phenolics was measured at 765nm. Standard gallic acid solutions were used for calibration curve and results were expressed as gallic acid equivalent per gram of extract (mg GAE/g).

Total antioxidant capacity

Sample (0.3 ml) was mixed with 3.0 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 min. Absorbance of all the sample mixtures was measured at 695 nm.[9] Total antioxidant capacity was expressed as ascorbic acid equivalent per gram extract (mg AAE/g).

Reducing power: Different concentration of extracts (50-500 µg/ml) in 1 ml of alcohol was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and 2.5 ml of 10% trichloroacetic acid was added. The reaction mixture was then centrifuged for 10 min. Further, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl3. The absorbance was measured at 700 nm.[10]

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

A 2 ml aliquot of solution was added to 2 ml of 2 x 10-4 mol/l ethanolic DPPH solution. The mixture was shaken vigorously and the absorbance was measured at 517 nm immediately. The decrease in absorbance was determined at 15 and 30 min until the absorbance reached a steady state (after nearly 30 min). The mixture with the addition of standard antioxidants served as a positive control.[11] All the tests were performed in triplicate, and the inhibition rate was calculated according to the following formula: % Inhibition of DPPH free radical = [(Ablank – Asample) / Ablank] x 100

ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid] scavenging activity

ABTS free radical was produced by reacting 7mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 16 h at room temperature.[12] Prior to assay, the solution was diluted in ethanol and equilibrated at 30°C to give an absorbance of 0.700 ± 0.02 at 734 nm. The stock solution of the sample extracts were diluted such that after introduction of 10 µl aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 ml of diluted ABTS solution to 10 µl of sample or Trolox standards in ethanol, absorbance was measured exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay and the percentage inhibition of the blank absorbance was calculated at 734 nm. Triplicate determinations were made at each dilution of the standard and percentage inhibition calculated and plotted as a function of Trolox concentration. The antioxidant activity is expressed as trolox equivalent antioxidant capacity (TEAC).

Ferrous ion chelation

Extract/standard (1 ml) was incubated with 0.5 ml of ferrous chloride (1 mmol). The reaction was initiated by the addition of 1 ml of ferrozine (5 mmol) and the total reaction volume was adjusted to 4 ml with ethanol. After the mixture had reached equilibrium (10 min), the absorbance was measured at 562 nm.[13] The negative control was prepared without the extract and EDTA was used as the positive control. The chelating activity of the extract on Fe2+ was calculated as follows

Chelating activity (%) = [(Acontrol/Asample) -1] x 100.

RESULTS AND DISCUSSION

Total phenolic contents: Folin Ciocalteau method for determination of phenolic content is based on oxidation of phenol by molybdotungstophosphoric reagent to yield a colored product that can be quantified by measuring absorbance at 765 nm.[14] Gallic acid was used as reference standard and the phenolic content of the extracts was expressed in mg Gallic acid equivalents per gram of extract (Table 1)**.** Ethyl acetate extract of *S. chrysanthemoides* demonstrated the presence of highest phenolic contents followed by methanolic and petroleum ether extract. The high amount of phenolics in ethyl acetate and methanolic extracts suggested the possible antioxidant potential of the *S. chrysanthemoides* extracts.

Total antioxidant capacity*:* Total antioxidant capacity determination by phosphomolybdenum method is based on the reduction of molybdenum VI (Mo6+) to form a green phosphate/Mo5+ complex at acidic pH which can be estimated by measuring absorbance at 695 nm.[15] Ascorbic acid was used as a standard to express the antioxidant capacity of extracts. The antioxidant capacity expressed as ascorbic acid equivalent (AAE mg/g extract) of ethyl acetate and methanolic extracts are 37.8 and 23.6 respectively. The ethyl acetate extract demonstrated higher total antioxidant capacity in comparison to methanolic extract.

Reducing power*:* Reducing power assay is based on the ability of extracts/reference compounds to reduce a yellow color Fe3+/Ferric cyanide complex to form Fe2+-ferrous complex which is estimated by measuring absorbance at 700nm.[16] A higher value of absorbance indicates higher reducing power of the extract. The methanolic extract demonstrated significant reducing power in the concentration range of 100-600µg/ml. The correlation between polyphenolic components and reducing power has been reported for several plant extracts. The results also indicate that methanolic extract of *S. chrysanthemoides*

consists of chemical structures with fair ability to donate electrons and convert reactive free radicals into stable products.

DPPH free radical scavenging ability: DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical changes its color from purple to yellow in the presence of antioxidant and is one of the most widely used to evaluate the antioxidant potential of extracts. Scavenging of the DPPH radical is also linked to the inhibition of lipid peroxidation. The results of the assay demonstrated antioxidant activity of extracts suggesting that the extracts are capable of donating hydrogen and acting as natural antioxidants. The ethyl acetate extract was potent in scavenging DPPH radical in comparison to methanolic extract. The radical scavenging ability was significantly low when compared to synthetic antioxidants like ascorbic acid and rutin. The potential to scavenge DPPH radical was measured by determining IC50 value which indicate the concentration required to inhibit 50% of DPPH free radicals. IC50 value of the ethyl acetate extract (264µg/ml) was lower than that of methanolic extract (376µg/ml) of *S. chrysanthemoides*. Lower value of IC50 indicates higher potency to scavenge DPPH free radicals.

ABTS radical cation scavenging assay: ABTS is a stable radical cation with blue color and characteristic absorption at 734nm. In this method an antioxidant is added to preformed ABTS radical cation and after a fixed time period the remaining ABTS is quantified spectrophotometrically.[17] Trolox (6-hydroxy-2, 5, 7, 8 -tetramethylchroman-2-carboxylic acid) a water soluble analog of vitamin E is used as standard to represent the antioxidant strength of sample as trolox equivalent antioxidant capacity (TEAC). TEAC is defined as micromolar trolox solution having an antioxidant capacity equivalent to 1g extract. The extracts exhibited good ABTS radical scavenging ability and were capable of decolorizing the ABTS radical. Trolox equivalent antioxidant capacity for methanolic extract and ethyl acetate are 7453.2 and 11547.6 respectively.

Ferrous ion chelation: Elemental species such as ferrous iron (Fe2+) can help in production of reactive oxygen species (ROS) within body and accordingly the ability of substances to chelate iron can be a measure of its antioxidant capacity.[18] The *S. chrysanthemoides* extracts were also evaluated for iron (II) chelating ability in the concentration range of 100-800µg/ml. At highest concentration the methanolic and ethyl acetate extract inhibited 52% and 40% of ferrous ion respectively. The chelating activity of the extracts was higher than rutin and significantly lower than the standard EDTA.

Table. 1: Total phenolic contents and antioxidant potential of *S. chrysanthemoides* extracts.

Extract/ Standard	TPCa (mg GAE/g)	TAOCb (mg AAE/g)	DPPH IC50(µg/ml)	ABTSc (TEAC)
PESA	113	ND	ND	ND
EASA	306	37.8	376	11547.6
MESA	284.4	23.6	264	7453.2
Rutin	-	-	45	-
Ascorbic acid	-	-	21	-

CONCLUSIONS

The extracts of *S. chrysanthemoides* were evaluated for the presence of total phenolic contents and thereafter tested for antioxidant potential. The ethyl acetate and methanolic extracts demonstrated the presence of fair amount of total phenolic contents and were further evaluated for antioxidant potential. The ethyl acetate extract exhibited higher total antioxidant capacity than methanolic extract. The methanolic extract exhibited highest reducing power in the concentration range of 100-600µg/ml. The extracts demonstrated fair ability to scavenge free radicals; the ethyl acetate extract demonstrated higher potency in comparison to methanolic extracts in both DPPH and ABTS free radical scavenging assays. The methanolic extract exhibited higher ferrous chelating activity than ethyl acetate extract in the concentration range of 100-800µg/ml. The antioxidant potential exhibited by the extracts was low when compared to standard antioxidants like rutin and ascorbic acid. The results are encouraging as these are obtained by crude extracts. In conclusion, the polar extracts of *S. chrysanthemoides* demonstrated antioxidant potential and suggest a need for phytochemical analysis of the polar extracts.

REFERENCES

1. Soukup, J., Vocabulario de los Nombres Vulgares de la Flora Peruana y Catalogo de los Generos, Editorial Salesiana: Lima, 1987; 393–394.
2. Villagrán, C., Castro, V., Sánchez, G., Romo, M., Latorre, C., Hinojosa, L. F. Estudios Atacameños. 1998; 16: 7-105.
3. El-Shazly, A., Doral, G., and Wink, M. Chemical composition and biological activity of essential oils of Senecio aegyptitus var. discoideus Boiss. Verlag der Z. Naturforsc C, 2002; 57: 434-439.

4. Hammond, G. B., Fernandez, I. D., Villegas, L. F. and Vaisberg, A. J., A survey of traditional medicinal plants from the Callejon de Huaylas, J. Ethnopharmacol, 1998; 61: 17-30.
5. Uzun, E., Sariyar, G., Adsersen, A., Karakoc, B., Otuk, G., Oktayoglu, E. and Pirildar, S., Traditional medicine in Sakarya Province (Turkey) and antimicrobial activities of selected species, J. Ethnopharmacol, 2004; 95: 287-296.
6. Adams, R. P., Identification of essential oil components by Gas chromatography/ Quadrupole Mass spectrometry. Allured Publ. Corp., Carol Stream, IL, U.S.A., 2004.
7. Gaur RD. Flora of district Garhwal north-west Himalayas, 1st ed.; Trans Media: Srinagar Garhwal, 1999.
8. Mbaebie BO et al. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of Schotia latifolia Jacq. Asian Pac J Trop Biomed, 2012; 118-24.
9. Prieto P et al. Spectrophotometric quantification of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to determination of Vitamin E, Anal Biochem, 1999; 269: 337- 41.
10. Oyaizu M. Studies on product browning reaction prepared from glucose amine. Japan J Nutri, 1986; 44: 307-15.
11. Sheng JC et al. Antioxidant activity of ethanol and petroleum ether extracts from Brazilian propolis. Eur Food Res Tech., 2007; 225: 249-53.
12. Roberta R et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Rad Bio Med., 1999; 26: 1231-37.
13. Wang T et al. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem., 2009; 116: 240-48.
14. Yuan Z, Wang Z. Phenolic composition and antioxidant activities of two Phlomis species: A correlation study. C R Biologies., 2009; 332: 816-26.
15. Jingsheng H et al. In vitro and in vivo antioxidant activity of the ethanolic extract from Meconopsis quintuplinervia. J Ethnopharmacol, 2012; 141: 104-10.
16. Kaviarasan S et al. In vitro studies on antiradical and antioxidant activities of fenugreek (Trigonella foenum graecum) seeds. Food Chem., 2007; 103: 31–7.
17. Chi-Chun W et al. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem., 2006; 97: 705-11.
18. Marcio C, Isabel CR. A review on antioxidants, proxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol, 2013; 51: 15-25.