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Antioxidant Potential of Extracts of *Artimisia scoparia:* Expression *In-vitro* Studies

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Abstract: Artimisia scoparia is extensively used as a natural therapeutic agent in the treatment of various diseases. The current study was designed to explore antioxidant potential of extract/fractions in DPPH free radical and reducing power assays. The results suggested marked antioxidant potential of extract/fractions in both DPPH and reducing power assays. Of the test articles, ethyl acetate was the most dominant fraction in both assays followed by crude methanolic extract. In conclusion, *A. scoparia* illustrated an outstanding antioxidant activity.

Key words: *Artemisia scoparia* · Extract/Fractions · DPPH and Reducing Power Assay

INTRODUCTION

The genus *Artimisia* L. represent one of the most largest and diverse genera of *Asteraceae*; consists of a approximately 522 species found throughout northern half of the world [1]. The aerial parts of the plant have been widely used for their hypoglycemic, hypolipidaemic, diuretic, antiulcer and anti-inflammatory activities [2], antiseptic, antibacterial, cholagogue, diuretic, purgative and vasodilator properties [3,4]. The essential oils of *A. scoparia* showed strong insecticidal activity against stored-product insects. Similarly, the aerial parts of plant exhibited significant anti-malarial, free radical scavenging and insecticidal activities [5] and the urease inhibitory profile is also observed [6].

Phytochemical analysis of the genus led to the isolation of several coumarins, flavonoids, phenylpropanoids, sterols and terpenoids (specially sesquiterpenes and monoterpenes) and their glycosides [5]. Keeping in view the diverse therapeutic uses of *A. scoparia*, the current study was designed to investigate the antioxidant potential of the plant in different *in-vitro* antioxidant paradigms.

MATERIALS AND METHODS

Plant Material: Fresh plant of *Artemisia scoparia* was collected from Parachinar Valley, Pakistan. The taxonomic identity of the plant was determined by a qualified plant taxonomist at department of Botany Kohat University of Science and Technology, Pakistan. The plant was washed 2-3 times with running tap water followed by shade-drying. The plant material was powdered for extraction.

Preparation of Solvent Extraction: Two kilograms of the shade dried, powder of plant materials were soaked separately in methanol for 10 days, extracted three times at room temperature in the same solvent and then filtered. The diluted extracts were concentrated on the vacuumed rotary evaporator under reduced pressure at a temperature of 46°C to give a residue (extract), which was further suspended in water and partitioned with different solvents (*n*-hexane, chloroform, ethyl acetate) to get their respective fractions. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these extracts/fractions as stored in airtight bottles for further use.

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Dpph Radical Scavenging Assay: The antioxidant activity was performed by DPPH radical scavenging assay. The electron donation abilities of the corresponding compounds and standards drug (Vitamin C) were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). All Analysis was carried out in triplicate according to the standard procedure [7-8]. Briefly, a 1 mM solution of DPPH radical solution in methanol was prepared and 1mL of this solution was mixed with 3 mL of sample solutions in methanol (containing 5-100 µg/mL) and control. The solution was stand for 30 min, in dark the absorbance value was monitored by using spectrophotometer at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follows

$$\% \text{ DPPH} = \frac{(\text{OD Control OD sample})}{\text{OD control}} \times 100$$

Where, OD control is the absorbance of the blank sample and OD sample is the absorbance of samples or standard sample. IC_{50} was the determined as 50% scavenging of test compounds on DPPH.

Reducing Power Assay: For reducing power assay, extract/fractions (10-100 µg/mL), phosphate buffer (2 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2 mL, were mixed and then incubated at 50°C for 20 min. Trichloroacetic acid (2 mL) was added to the mixture. A volume of 2 mL from each of the aforementioned mixtures was mixed with 2 mL of distilled water and 0.4 mL of 0.1% (w/v) ferric chloride in a test tube. After 10 min reaction, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated a high reducing power. Butylated hydroxytoluene (BHT) was used as reference standard. IC_{50} was the determined as 50% scavenging of test compounds [9].

Statistical Analysis: The resulting data were expressed as the mean ± SEM (n=3) in each group. To determine the differences between groups, one-way analysis of variance (ANOVA) was performed (GraphPad PRISM 6, San Diego, CA, USA) using the least significant difference (LSD) test at P<0.5 or P<0.01.

RESULTS

Effect of Extract/fractions in Dpph Free Radical Assay: The percent effect of free radical scavenging activity against DPPH in comparison with the standard drug, ascorbic acid is illustrated in figure 1. The crude extract caused concentration dependent scavenging effect with maximum of 79.55% at 100 µg/ml (Fig. 1a) with the estimated IC_{50} value of 61.5 µg/ml. Upon fractionation, n-hexane exhibited least free radical quenching effect with maximum of 27.49% at 100 µg/ml (Fig. 1b). The chloroform fraction elicited considerable scavenging effect with maximum of 57.34% at 100 µg/ml (Fig. 1c) with IC_{50} value of 61.5 µg/ml. 88.7 µg/ml. Of the fractions, ethyl acetate was the most dominant with maximum of 90.20% at 100 µg/ml (Fig. 1d) with IC_{50} value of 45.10 µg/ml. In case of aqueous fraction, the percent free radical effect was 59.42% and IC_{50} value of 84.05 µg/ml.

Effect of Extract/fractions in Reducing Power Assay: The extract/fractions of *A. scoparia* provoked marked scavenging effect reducing power assay in a concentration-dependent manner (Fig 2). The crude extract produced 81.05% quenching at 100 µg/ml with IC_{50} value of 59.684.05 µg/ml (Fig 2a). When fractioned, ethyl acetate was the most potent fraction with 91.20% quenching effect at 100 µg/ml and IC_{50} value of 43.03 µg/ml (Fig 2d) followed by aqueous fraction with 62.43% at 100 µg/ml with IC_{50} value of 72.13 µg/ml (Fig 2e). The *n*-hexane was least active followed by chloroform (Fig 2b, c).

DISCUSSION

Free radicals are produced in several oxidative-reductive processes over expression of which may provoke oxidative damage in various human components (e.g., lipids, protein and nucleic acids). This may also contribute in different processes that cause mutations. Furthermore, free radical reactions may involved or augment several chronic diseases such as cancer, hypertension, heart disease, rheumatism, cataracts etc. that affect life style [10]. On the basis of their mode of action, antioxidants may be classified as free radical terminators, chelators of metal ions involved in catalyzing lipid oxidation or oxygen scavengers that react with oxygen closed system [11].

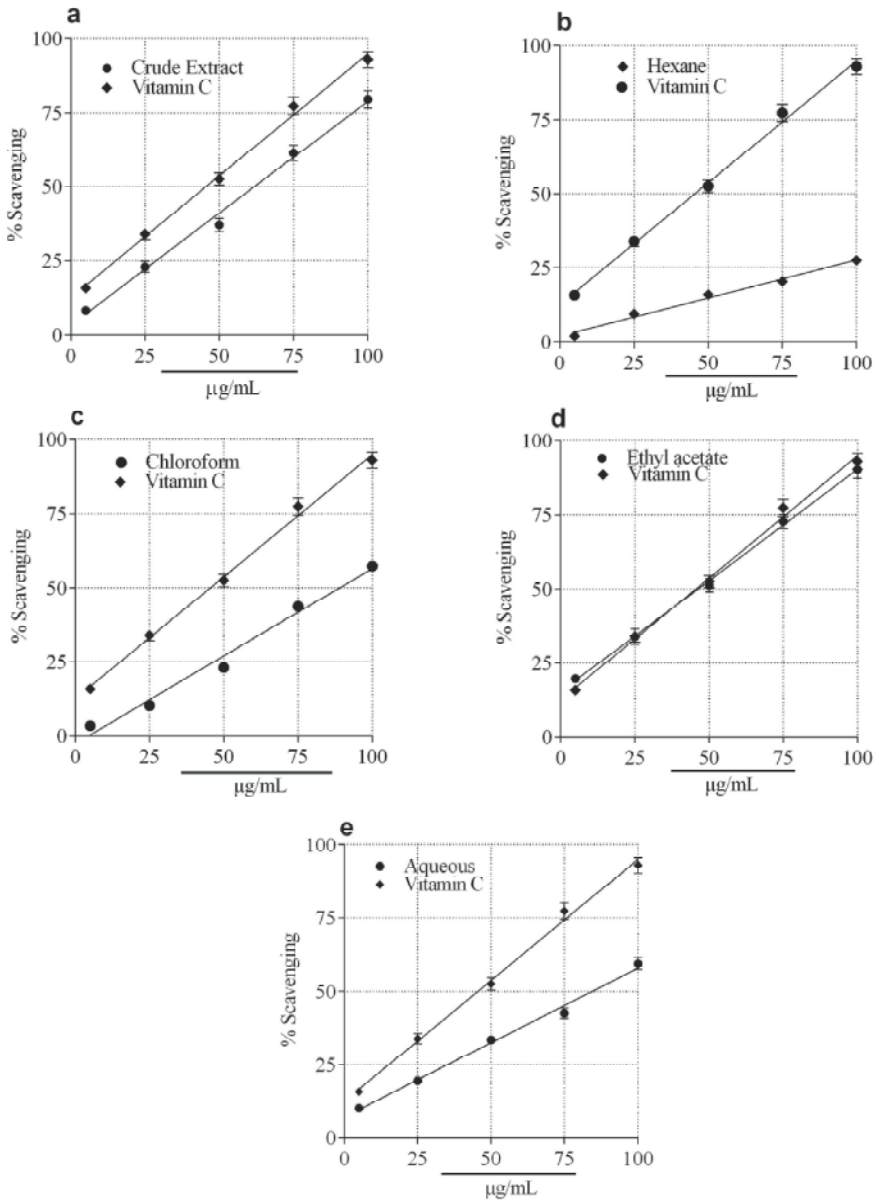

Fig. 1: Percent free radical scavenging effect against DPPH [a] crude extract, [b] n-hexane, [c] chloroform, [d] ethyl acetate and [e] aqueous fraction. The resulting data were expressed as the mean ± SEM (*n*=3) in each group.

By inhibiting various oxidizing chain reactions, antioxidants are free radical scavenger's which offer defense to human body against oxidative damage. When these constituents are present at low concentration in body they stop the oxidation of an oxidizable substrate [12,13]. These antioxidants play important roles in delaying the development of chronic disorders such as cardiovascular diseases, cancer, atherosclerosis and inflammatory diseases [14,15].

The natural antioxidants play a principal role in helping endogenous antioxidants to neutralize oxidative stress. Several epidemiological, clinical and experimental data suggest that plant based antioxidants have beneficial effects on prevention on chronic diseases [16-18]. As a result, there has been a keen interest in evaluating the role bioactive constituents from medicinal plants in reducing the risk of the aforesaid diseases.

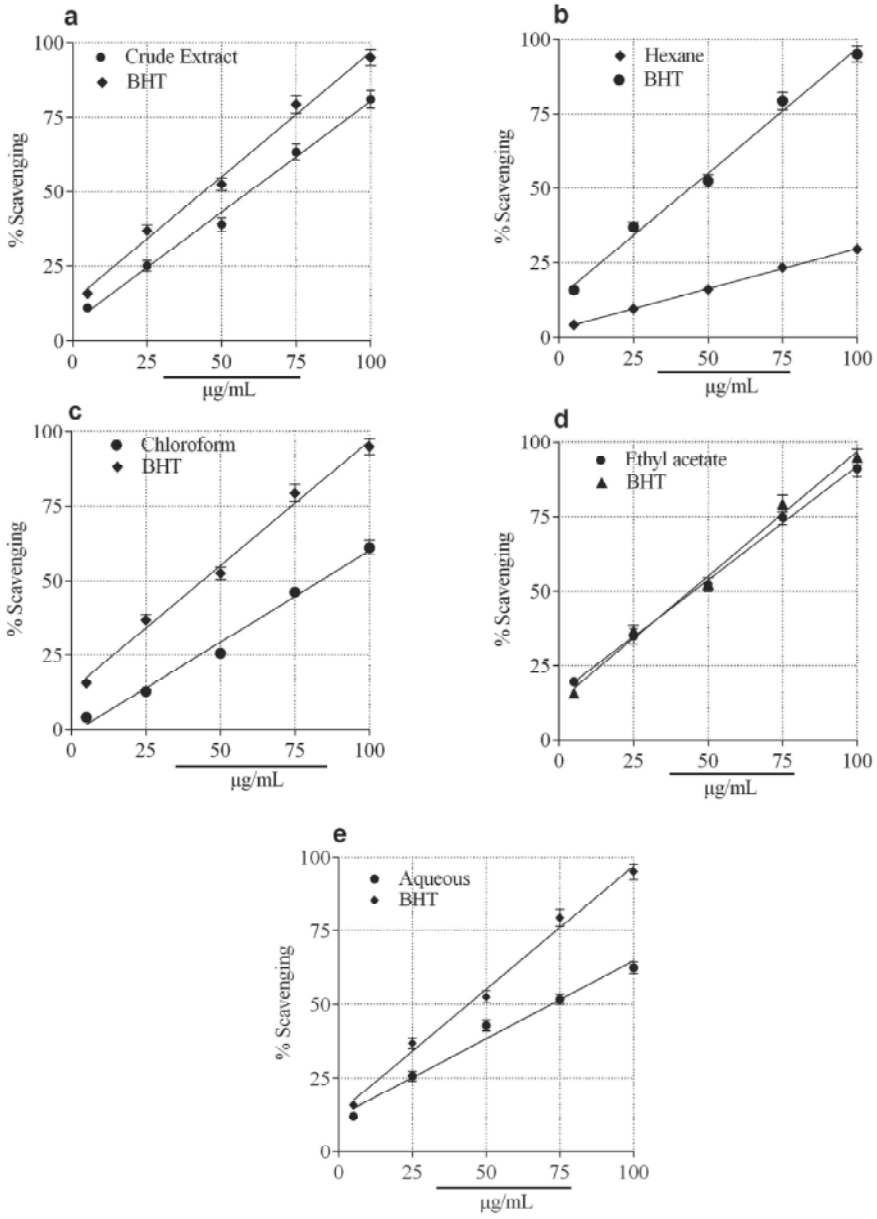

Fig. 2: Percent effect in reducing power assay of [a] crude extract, [b] n-hexane, [c] chloroform, [d] ethyl acetate and [e] aqueous fraction. The resulting data were expressed as the mean ± SEM (n=3) in each group.

There are several methods for the estimation of free radical scavenging activity but DPPH and reducing power assays have gained tremendous reputation in recent times due to simplicity and easiness of these in-vitro screening methods. It is also found rapid and sensitive techniques when employed for the assessment of plants extracts. In the present study, the extract/fractions of *A. scoparia* evoked marked attenuation of free radical tendency of DPPH and reducing power assays.

To sum-up, the extract/fractions of *A. scoparia* exhibited marked attenuation of free radical potential of DPPH and reducing power assay and thus offer potential natural antioxidant. In this connection, isolation of bioactive constituents from the plant would enable to us to declare chemical background of our finding.

Conflict of Interest: The authors of this article have no conflict of interest.

REFERENCES

1. Mohsen, H. and F. Ali, 2008. "Study of genetic polymorphism of *Artemisia herba-alba* from Tunisia using ISSR markers," African Journal of Biotechnology,7(1): 44-50.
2. Author ed.^eds., "Medicinal Plant," Tehran, Iran.: University of Tehran Press, 1990, p.^pp. Pages.
3. Safaei-Ghomi, J., A. Bamoniri, M. Sarafraz and H. Batooli, 2005. "Volatile components from *Artemisia scoparia* Waldstet Kit growing in central Iran," Flavour and Fragrance Journal, 20: 650-652.
4. Mirjalili, M., S. Tabatabaei, J. Hadian, S. Nejad-Ebrahimi and A. Sonboli, 2007. "Phenological variation of the essential oil of *Artemisia scoparia* Waldst. et Kit. from Iran," Journal of Essentional Oil Research, 19: 326-329.
5. Afshar, F.H., A. Delazar, O. Janneh, H. Nazemiyeh, A. Pasdaran, L. Nahar and S.D. Sarker, 2011. "Evaluation of antimalarial, free radicalscavenging and insecticidal activities of *Artemisia scoparia* and *A. spicigera,* Asteraceae," Brazilian Journal of Pharmacognosy, 21(6): 986-990.
6. Khan, M.A., H. Khan, S.A. Tariq and S. Pervez, 2014. "Urease inhibitory activity of aerial parts of *Artemisia scoparia*: Explorationinan *in vitro* study," Ulcers, vol. Volume 2014, Article ID 184736, 5 pages, pp: 1-5.
7. Khan, H., M. Saeed, M.A. Khan, K. Inamullah, M. Ahmad. M. Naveed and A. Khan, 2012. "Antimalarial and free radical scavenging activities of rhizomes of *Polygonatum verticillatum* supported by isolated metabolites," Medicinal Chemistry Research, 21(7): 1278-1282.
8. Khan, H., M. Saeed, N. Muhammad, A. Rauf, A.Z. Khan and R. Ullah, 2013. "Antioxidant profile of constituents isolated from *Polygonatum verticillatum* rhizomes," Toxicology and Industrial Health vol. DOI:10.1177/0748233713498454.
9. Muhammad N. and M. Saeed, 2011. "Biological screening of Viola betonicifolia Smith whole Plant," African Journal of Pharmacy Pharmacology, 5: 2323-2329.
10. Rauf, A., G. Uddin and H. Khan, 2014. "Preliminary antioxidant profile of *Pistacia integerrima* Stewart whole plant," Pakistan Journal of Pharmaceutical Sciences, vol. Manuscript No.2405…pages?.
11. McDowell, A., S. Thompson, M. Stark, Z-Q, Ou and K.S. Gould, 2011. "Antioxidant Activity of Puha (*Sonchus oleraceus* L.) as Assessed by the Cellular Antioxidant Activity (CAA) Assay," Phytotherapy Research, 25(12): 1876-1882.
12. Khan, H., M.A. Khan and Abdullah, 2012. "Antibacterial, antioxidant and cytotoxic studies of total saponin, alkaloid and sterols contents of decoction of Joshanda: Identification of components identification through thin layer chromatography," Toxicology and Industrial Health, vol. DOI: 10.1177/0748233712468023.
13. Raziq, N., N. Muhammad, K. Chishti, M. Saeed, S. Rahman and Khan, 2011. "Correlation of the antioxidant capacity with the phenolic contents of *Hypericum monogynum* and *Hypericum perforatum*," African Journal of Pharmacy and Pharmacology, 5: 1872-1876.
14. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. "Antioxidant activity applying an improved ABTS radical cation decolorization assay," Free Radical Biology and Medicine, 26(9-10): 1231-1237.
15. Uddin, G., A. Rauf, M. Arfan, M. Ali, M. Qasir, M. Saadiq and M. Atif, 2012. " Preliminary phytochemical Screening and antioxidant activity of *Bergenia caliata*," Middle-East Journal Scientific Research, 11(8): 1140-1142.
16. Khan, H., M. Khan, N. Muhammad, N. Ashraf, F. Gul and S. Tariq, 2012. "Antiinflammatory and antioxidant activity of Joshanda partially mediated through inhibition of lipoxygenase," Phytopharmacology, 3: 19-28.
17. Lateef, M., L. Iqbal, N. Fatima, K. Siddiqui, N. Afza, M. Zia-ul-Haq and M. Ahmad, 2012. " Evaluation of antioxidant and urease inhibition activities of roots of *Glycyrrhiza glabra*," Pakistan Journal Pharmaceutical Sciences, 25: 99-102.
18. Loizzo, M., R. Tundis, U. Hawas, K. Rashed, F. Menichini, N.G. Frega and F. Menichini, 2009. "Antioxidant and Antiproliferative Activity of *Diospyros lotus* L," Plant Foods Human Nutrition 64: 264-270.