J. Indian bot. Soc. (2024) Vol. 104 (1): 35-40

e-ISSN:2455-7218, ISSN:0019 - 4468

https://doi: 10.61289/jibs2024.10.31.1197

www.indianbotsoc.org

RESEARCH ARTICLE

Screening antioxidant activity in different parts of plant *Achyranthes aspera* L.

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Abstract

Achyranthes aspera L. (Amaranthaceae) is an annual or perennial native herb that is found all over the Indian subcontinent, usually growing along roadsides or in wastelands of tropical and subtropical continents including Asia, Africa, and America. It is a medicinal weed plant possessing pharmacological potential as a purgative, laxative, mild astringent in bowel issues, anti-fertility agent, antibacterial, anti-inflammatory, antiarthritic, anti-obesity, hypoglycaemic and wound healing properties, etc. that are inferring towards the potential application of the tested plant part extracts in pharmaceutical industries for manufacturing drugs employed in treatment of various human ailments. The methanol extract of (*A. aspera* L.) root, stem, leaves, and inflorescence was tested for the antioxidant potential against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and FRAP showing their reducing power (Fe^{3+}— Fe^{2+} Transformation ability). The current investigation however showed relatively lower hydrogen donating potential than the standard ascorbic acid (AA). The relatively strong hydrogen donating and radical scavenging potential of the methanol extract of both root and stem with a comparatively lower IC50 than the antioxidant potential of the inflorescence with a much higher IC50. On the other hand, roots of the plant showed through FRAP, relatively higher reduction potential in the methanol extract of the root and the least FRAP value was observed in the stem extract of the plant. This study provides evidence to substantiate traditional use of the plant for its hydrogen donating as well as the ferrous ion reducing potential.

Keywords: *Achyranthes aspera*, GC-MS, Phyto Composition, Antioxidant, DPPH, Free Radical scavenging, FRAP.

Introduction

The reactive oxygen species (ROS), which are also known as prooxidants, are characteristic aspect of a normal aerobic organism. The term reactive oxygen species (ROS) collectively describes radicals that are oxygen-centered such as hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot}$) and some non-radical oxygen-derived species: singlet oxygen (½ O_2), hydrogen peroxide (H_2O_2), etc. The regulation of homeostasis in biological systems relies greatly on these radical reactions. Free radicals perform a wide range of physiological processes, including oxidative transformation reactions mediated by cytochrome P_{450}, a variety of enzymatic oxidation reactions, oxidative phosphorylation, control of smooth muscle tone, and microbial death (McCord 2000, Sokmen *et al.* 2004, Singh and Marimuthu 2006).

Excessive free radical production causes negative biological implications by oxidizing biomolecules (for example, protein, amino acids, lipids, and DNA), resulting in cell injury and death, and a variety of pathological events (McCord 2000; Halliwell *et al.* 1990; Halliwell *et al.* 1992). Free radical production has been linked to a variety of diseases conditions that ranges from an immune/inflammatory distress to serious health issues like cancer and coronary thrombosis. To mitigate the damaging effects of ROS, organisms have evolved defense mechanisms known as antioxidants. They are categorised as chemicals that, when added to the food products, particularly lipids or lipid-containing foods, helps in extending the shelf life of the concerned food product. Its mechanism is based on

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How to cite this article: Mishra A, Khangarot K, Bhardwaj R, Sharma RA. (2024). Screening antioxidant activity in different parts of plant *Achyranthes aspera* L. J. Indian bot. Soc., 104 (1): 35-40. Doi: 10.61289/jibs2024.10.31.1197

Source of support: Nil

Conflict of interest: None.

Received: 20/05/2023 **Accepted:** 31/10/2023 **Published online**: 25/01/2024

the prevention of lipid peroxidation, that functions as the principal cause of food spoilage during the stages of food processing and storage (Singh and Marimuthu 2006).

Since the start of the 20th century, synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG) are some of the most commonly used antioxidants. However, due to their potential carcinogenic effect on human health, limitations are being placed on the usage of these substances (Wanasundara 1998). As a result, it has become necessary to search for alternate, natural and secure sources of relatively safer antioxidants or the ones that impart relatively milder side-effects and the hunt for such natural antioxidants have brought forward the proposition of extracting active biochemicals with antioxidant potential from the plants. There has been a noticeable increase in the research of antioxidants screening as well as their extraction from natural origin in recent years (Madhav and Salunkhe 1905, Goli *et al.* 2005, Lòpez *et al.* 2007, Yasoubi *et al.* 2007, Chohan *et al.* 2008,)

Achyranthes aspera L. (Figure 1) is an annual or perennial herb in the Amaranthaceae family that is a weed plant, commonly called Prickly chaff flower or by local names like Chirchita, Latjeera (Hindi) and Apamarga (Sanskrit). The plant is a native herb that is found growing wild abundantly over the Indian subcontinent at an altitude of 2100 m as well as in South Andaman Islands, usually growing along roadsides or in wastelands, plains, foothills, abandoned gardens, crops, grasslands, and forest margins of tropical and warmer regions and is found distributed throughout tropical portions of the continents including Asia, Africa, Australia, and America (Barua *et al.* 2012).

The plant *A. aspera* has been reported for various pharmacological activities such as anti-leprotic (Ojha *et al.* 1966), abortion-inducing (Pakrashi 1977), contraceptive (Wadhwa *et al.* 1986), antibacterial (Verma *et al.* 1997), thyroid-stimulating, *in-vitro* antiperoxidative effect (Tahiliani *et al.* 2000), cancer chemoprotective (Chakraborty *et al.* 2002), stimulates reproductive functions (Sandhyakumary *et al.* 2002), anti-arthritic, anti-inflammatory activity (Gokhale *et al.* 2002) and hepatoprotective potential (Bafna and Mishra 2004). Root extract is used in the treatment of conditions like diabetes (Akhtar and Iqbal 1991), asthma hypertension and malarial fever (Neeru and Sharma 2006). Previous research has reported phosphorylase activity in the cardiac tissues from the extracted saponins of this plant (Ram *et al.* 1971). The decoction of the whole plant has been reported to exhibit diuretic properties and was reported as an anti-pneumonial agent (Neeru md Sharma 2006). Preliminary phytochemical analysis on *A. aspera* L. following standard procedures reported the presence of several classes of phytochemicals like flavonoids, alkaloids, terpenoids, saponins, steroids, quinones, acyl quinic acid, essential oils, glycosides, fatty acids and esters (Chakraborty *et al.* 2002, Vetrichelvan and Jagadeesan 2003, Edwin *et al.* 2008, Gawande *et al.* 2017).

Figure 1: Inflorescence of the *Achyranthes aspera* plant

The aims of this work were: (i) to determine the hydrogen donating potential by using the DPPH (2, 2, diphenyl 1 picrylhydrazyl) radical scavenging assay and (ii) analyzing the antioxidant activity by evaluating the ferric ion reduction potential of the methanol extract of root, stem, and inflorescence of *A. aspera* L.

Material and methods

Chemical Reagent

All the chemicals employed in the analysis procedure were of analytical grade. Methanol, Sodium chloride, hydrochloric acid, disodium hydrogen phosphate, potassium chloride, potassium dihydrogen phosphate, potassium ferricyanide, from Merck, and a few chemicals like Tri-chloro Acetic acid (TCA), ferric chloride, ascorbic acid, DPPH, ethanol from Sigma-Aldrich. Distilled water was obtained from the steam distillation unit installed at the lab facility.

Collection of plant material

A. aspera fresh plant material of the plant was collected from Chandli village, Deoli tehsil of district Tonk in Rajasthan, India in July 2021. The collected plant material was thoroughly rinsed under a running tap in order to remove any dirt and debris. The plant material collected was blotted dry to remove any excess moisture. The inflorescence from the collected plants was clipped, collected and shade dried for a week, and ground into a uniform powder. The dried powder was then kept stored in airtight containers for future extractions.

Sample preparation

The soxhlet extracted ME of the inflorescence was allowed to dry completely so as all the solvent from the extract would be evaporated. Weigh the dried sample obtained and add the solvent (methanol) appropriately to make a stock solution of 100 µg/ml or 5000 µg/ml (Figure 2).

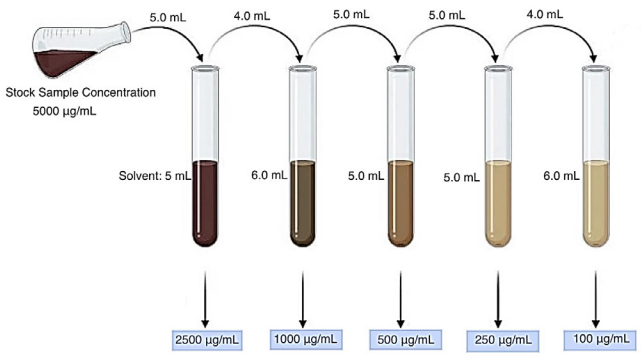

Figure 2: Sample preparation of concentration range 100 - 5000 µg/mL

2,2-diphenylpicrylhydrazyl (DPPH) assay

For analyzing the hydrogen atom donating ability, the methanol extract of various plant parts was tested by DPPH radical scavenging assay (Blois 1958, Desmarchelier *et al.* 5 µL of varying concentration (1 µg/mL to 1000 µg/mL) of the test extract was added to 0.2 ml of 0.2mM DPPH solution. The reaction was set in triplicate and each tube was incubated for 30 minutes in dark. In methanol solution, a nitrogen-centered free radical DPPH produces violet color. With the addition of a sample, the DPPH radicals were reduced, generating a yellow pigmented product that is diphenyl picryl hydrazine produced in accordance with the increasing concentration of the test extract. The polarity of the reaction medium, the radical scavenger's chemical makeup, the pH of the reaction mixture, sample concentration, and reaction duration all affect DPPH scavenging activity (Ayres 1949, Sloane and William 1977). Afterward, the extent of decolorization was observed via a UV-Visible spectrophotometer at 517 nm wavelength using a microplate reader (iMark, BioRad). The reaction mixture containing 20 µL of deionized water served as the negative control in the conducted assay. The scavenging activity was determined as '% inhibition' with respect to the control. The higher % of radical scavenging activity is indicated by the value of lower absorbance of the reaction mixture. AA was employed as a reference in the assay and was dissolved with methanol to prepare a stock solution of a similar concentration range as the test extract. The percentage DPPH free radical scavenging effect was enumerated applying the provided equation:

%Scavenging Activity

The IC50 values of the tested extract was calculated from the linear equation obtained from the respective inhibition curves prepared from the exhibited absorbances of the extracts and is determined as the concentration of the test sample that exhibits exactly half of the maximal radical scavenging percentage exhibited.

Ferrous reducing antioxidant potential (FRAP) assay

For analyzing the potent ferrous reducing antioxidant activity, the method provided by Oyaizu (Oyaizu 1986) was employed. 400 µL of 0.2 M sodium phosphate buffer with pH 6.6 was prepared by mixing 0.2 gm - potassium dihydrogen phosphate, 1.44 gm - disodium hydrogen phosphate, 0.2 gm - potassium chloride, 8 gm - sodium chloride in a 1000 mL standard flask. Deionized water (800 mL) was added to the flask, followed by adjusting the pH of the resultant mixture obtained to 6.6 by adding hydrochloric acid to the test mix, then the volume of the resultant mixture has been adjusted with deionized water and 0.5 mL of 1% potassium ferricyanide solution. Afterward, 100 µL of test extracts of diverse concentration range (1 µg/mL to 1000 µg/mL) was added to the resultant mix. The mixture was well vortexed followed by its incubation for 20 minutes at 50°C. After incubation to the mixture, 0.5 mL of trichloroacetic acid was added followed by centrifugation of the resultant mixture for a short time period of 10 minutes at 5000 rpm. The supernatant separated by centrifugation was collected and mixed with 100 µL of double distilled or deionized water and 50 µL of ferric chloride (0.1%). The colored solution obtained was observed for their individual absorbance values at 655 nm wavelength against the blank using a microplate reader (iMark, BioRad). A typical blank solution is composed of the same solution mixture without adding any of the tested plant extracts/standard and treated/incubated under similar lab conditions as the test extract.

Results

DPPH radical scavenging potential

The % yields of the scavenged products obtained from the radical scavenging assay of the methanol extracts of tested plant parts of *A. aspera* are shown in Table 1. The methanol extract of different plant parts exhibits considerably

Table 1: Estimation of free radical scavenging activity with the methanolic extracts of different parts of *Achyranthes aspera* L.

Contents	*Concentration (µg/mL)*	*O.D. (512 nm)*	*% DPPH scavenging activity*
Blank	-	0.036	-
Ascorbic acid	5000	0.119	89.87 ±
Root	5000	0.453	62.46 ± 5.347
Stem	5000	0.452	62.17 ± 5.394
Inflorescence	5000	0.853	26.89 ± 1.437

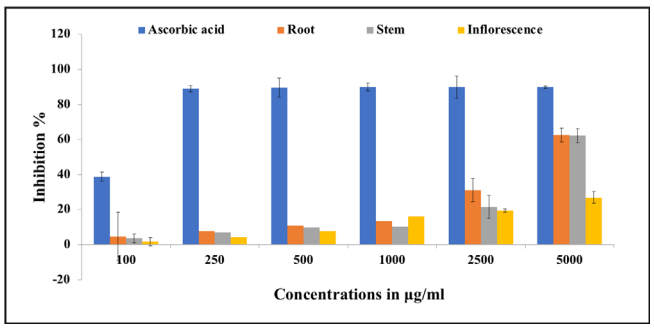

Figure 3: Valuation of radical scavenging potential at varying concentrations of different plant part extracts and standard ascorbic acid

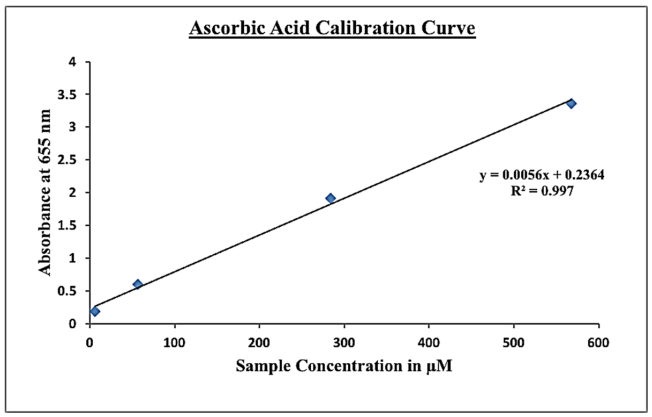

Figure 4: FRAP assay: Calibration Curve of standard ascorbic acid

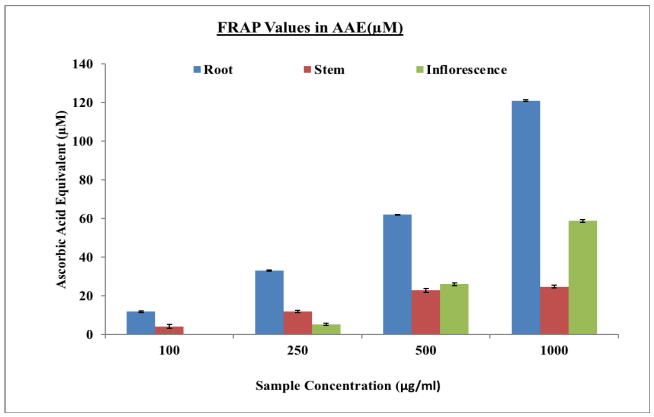

Figure 5: FRAP values of different plant parts of *Achyranthes aspera* L.

significant antioxidant activity when compared with standard AA. Figure 3 represents the comparative radical scavenging activity exhibited by the methanol extract of tested plant parts at a varying range of concentrations compared to the ascorbic acid (standard). All the tested plant parts have shown a progressive increase in the radical scavenging potential in a concentration-dependent manner (Halliwell and Gutteridge 1998).

Both root and stem methanol extracts exhibited relatively higher scavenging activity with an IC50 of 4177 µg/ml and 4334 µg/ml as compared to the methanol extract of inflorescence 9294 µg/ml IC50 value. This relatively higher hydrogen-donating potential of the root and stem might be due to the presence of relatively higher amounts of total polyphenols. The inflorescence extract showed the least scavenging activity among all the tested plant parts.

Compared with the published results on the methanol root extracts of *Achyranthes aspera*, the radical scavenging activity observed at 62.46 ± 5.347 % at 5000 µg/ml was significantly lower than the previously reported quenched DPPH of 77.6 ± 0.05 % at 250 µg/ml (Rama *et al.* 2013). The previous research findings of the IC50 value of 556.07 µg/ml reported from the leaves (Edwin *et al.* 2008) was significantly lower than the observed IC50 value of 4177 µg/ml. Previous biochemical study on the plant have reported range of phenolic compounds in the plant which include phenols, polypropenol, benzoquinones, phenolic quinones, flavonoids, isoflavonoids, anthraquinone, anthocyanidins, xanthones, lignins, melanins, tannins, etc. These compounds have been reported to exhibit free radical scavenging potential via single electron transfer (Capecka 2005).

Table 2: Attributes of standards used during FRAP experiments

Parameter	*Ascorbic Acid*
Concentration	5 — 575 µM
Calibration points	4
Linear equation	y = 0.005x + 0.236
R^2	0.951

Ferric ion reducing potential

The current research screened the presence of antioxidants that donates electrons and causes the reduction of Fe^{3+}—Fe^{2+}. The formation/intensity of Perl's Prussian blue color at 655 nm, which signals an increase in reducing potential, might then be used to monitor the amount of Fe^{2+} complex.

From the current analyses, it has been observed that the value of the ferrous reducing potential was found lower with respect to the standard AA. The characteristics of the standard used is provided in Table 2 and the plotted Standard calibration curve is provided in Figure 4. In the current evaluation, antioxidant activity is assessed based on its ability to convert ferric (III) iron to ferrous (II) iron. The results are given as µM AEAC (micromolar ascorbic acid equivalent antioxidant capacity). The antioxidant activity measured by FRAP was found to be positively related to the concentration of the extract. The absorbance exhibited by the root methanol extract at 100 µg/ml concentration was 0.295 which is greater than double the previously reported absorbance of 0.128 exhibited by the root methanol extract of the plant at 655 nm (Rama *et al.* 2013). The comparative FRAP activity exhibited by different plant part extract is represented in Figure 5.

The FRAP values calculated from the current research analysis of the ferric reduction potential were shown in Table 3. The root methanol extract of the plant was observed to exhibit most significant reduction potential with the observed maximum FRAP value of 121.1 ± 0.529 AAE (µM),

Table 3: Estimation of FRAP values in the methanolic extracts of different plant parts of *Achyranthes aspera* expressed as Ascorbic Acid Equivalent (µM)

Concentration of extracts (µg/ml)	*FRAP values in µM AEAC*		
	Root	*Stem*	*Inflorescence*
100	11.83 ± 0.416	4.17 ± 0.924	NA
250	33.1 ± 0.346	11.9 ± 0.6	5.17 ± 0.503
500	62.03 ± 0.115	22.83 ± 1.026	25.97 ± 0.702
1000	121.1 ± 0.529	24.7 ± 0.721	58.77 ± 0.757

followed by the inflorescence methanol extract with FRAP value of 58.77 ± 0.757 AAE (μM) at 1000 μg/ml concentration, and then least FRAP value in the stem extract (24.7 ± 0.721 μM AEAC) at 1000 μg/ml concentration. The findings of the conducted research were found to be in agreement with the previously reported FRAP analysis, which indicates towards the root exhibiting the strongest reduction potential (Upadhya 2015).

Conclusion

Considering the results obtained from the hydrogen donating and ferric reducing potential for screening of antioxidant activity, it can be concluded that the roots of plants exhibiting the most significant antioxidant activity as obtained from the results of both FRAP as well as DPPH assay. The stem of the plant has been observed with a relatively higher hydrogen atom donating potential than the methanol extract of the inflorescence. On the other hand, the ferric ion reduction potential has been observed to be relatively significant in the inflorescence extract rather than stem extract in each tested concentration. The current analysis results suggested significant antioxidant potential in the methanol extracts of root, leaf and inflorescence of *A. aspera* and therefore forms a potential natural source of antioxidant compounds for formation of therapeutic drugs.

Acknowledgement

The authors would like to express the deepest gratitude to the Head of the Department (Botany), Rajasthan University for providing research facilities, laboratory equipment, and ample knowledge of the conducted research work. We would also like to thank our supervisor Dr. R.A. Sharma and co-supervisor Dr. Richa Bhardwaj for their contribitions in the conducted research. We are also thankful for the helping aid and constant moyivation provided by the fellow research scholars.

Author contribution statement

The material collection, research experimentation, calculations and statistical analysis required for conceptualizing the results obtained of the conducted research has been done by the corresponding author Ashmita Mishra. The revision and final check of the statistical analysis results and the manuscript final editing has been done by Kiran Khangarot. Prof. R.A. Sharma and Asstt. Prof. Richa Bhardawaj has provided necessary guidance and inputs for the concerned research protocols followed in the presented research work. All the authors have discussed the required review to be considered of the concerned research, the methodology to be followed, the calculated results and the data interpretation and thereafter contributed the final manuscript.

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