ORIGINAL ARTICLE

Evaluation of quantitative and antioxidant activity of *Achyranthes aspera* roots and inflorescences

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Achyranthes aspera Linn. (Amaranthaceae family) is an important medicinal plant that possesses many therapeutic properties. The study was conducted to investigate the quantitative components, total antioxidant, and free radical scavenging activities of various extracts of roots and inflorescences of *A. aspera*. Present study was conducted to evaluate the antioxidant activity of different sequential extracts (petroleum ether, benzene, chloroform, ethyl acetate, ethanol, and distilled water) of *A. aspera* roots and inflorescence, which includes 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC). On other hand, quantitative assays (total phenol content, total flavonol content, total flavonoid content, total proanthocynidin content, and total tannin content) were also assessed using standard protocols. Results depicted strong antioxidant activity in both root and inflorescence parts of *A. aspera*. It can be inferred that *A. aspera* serves as a potent antioxidant agent and could be exploited to manufacture drug for the treatment of various human ailments.

Key words: *Achyranthes aspera, antioxidant, 1,1-diphenyl-2-picrylhydrazyl, ferric reducing antioxidant power, tannin*

INTRODUCTION

Reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism and they are well recognized for playing a dual role as both deleterious and beneficial species, as they can be either harmful or beneficial for living systems.[1] They cause the oxidative stress in the body when present in more amounts. Oxidative stress, induced by oxygen radicals, is believed to be a primary factor in various degenerative diseases, such as cancer,[2] atherosclerosis, gastric ulcer,[3] and other conditions. For the treatment of oxidative stress, commonly used antioxidants are butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ). These are chemical antioxidants that are normally not used due to many side effects and other problems such as low availability and high cost. So herbal (plant) products are the better option for the treatment against the oxidative stress. These are easily available and non-toxic.[4] Medicinal plants contain various bioactive compounds such as alkaloids, tannins, flavonoids, and phenolic compounds that are responsible for the treatment of various oxidative stress-related diseases.[2,5-7]

Achyranthes aspera Linn. of Amaranthaceae family is an important medicinal plant that possesses various bioactive molecules like phenol, flavonoids, tannins, alkaloids, saponins, terpinoids, and reducing sugars[8,9] and many activities such as antimicrobial,[10-14] antihypoglycemic,[15] hypolipidemic,[16] antihyperglycemic,[17] anticarcinogenic,[18] antiinflammatory,[19] cardioprotective,[20] hepatoprotective,[21] immunomodulatory,[22] and antiparasitic[23,24] activities. Keeping in view the aforementioned medicinal properties of the *A. aspera*, the present study was carried out to test the *in vitro* quantitative and antioxidant assays of the root and inflorescences of various extracts of *A. aspera*.

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MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in the present study were purchased from reliable firms like Merck, CDH, HI MEDIA and were of analytical grade.

Experimental plant

Roots and inflorescence of *A. aspera* were collected seasonally from Banasthali University, Rajasthan and were authenticated by Botanist of Krishi Vigyan Kendra of this University.

Preparation of different fractions of *A. aspera*

The plant parts (roots and inflorescences) were cleaned, dried, and powdered with the help of mixer grinder separately. Various extracts were prepared using sequential solvents from non-polar to polar (petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water). These extracts were concentrated using rotary evaporator and stored at 4°C in airtight containers for further experimental studies.

In vitro physicochemical quantitative assays

Determination of total phenol content

Total phenol content was determined by using Folin and Ciocalteu (1927) method.[25] First, prepared the various concentrations (100-1000 µg/ml) of extracts and standard (gallic acid). The reaction mixture contained 1 ml of Folin-Ciocalteu reagent and 4 ml of sodium carbonate, which was incubated for 2 h at room temperature and then centrifuged at 2000 g for 5 min. Absorbance of supernatant, was taken at 760 nm. Total content of phenol compounds in gallic acid equivalent (GAE) was calculated by the following formula:

$$C = c. V/m$$

Where C is the total content of phenol compounds, mg/g plant extract (GAE); c is the concentration of gallic acid established from the calibration curve (mg/ml); V is the the volume of extract (ml); m is the weight of pure plant extract (g).

Determination of total flavonoid content

The principle of estimating total flavonoid content, given by Ordon *et al*., (2006)[26] is also known as aluminum chloride colorimetric method. Different concentrations (100-1000 µg/ml) of extracts and standard (rutin) were prepared and then 0.5 ml of aluminum chloride was added, incubated for 60 min at room temperature and absorbance was taken at 420 nm. Total content of flavonoid compound was calculated by the following formula

$$X = (A. m_o)/(A_o. m)$$

Where X is the total flavonoid content, mg/mg plant extract in rutin equivalents (REs); A is the absorbance of plant extract solution; A_o is the absorbance of standard rutin solution; m is the weight of plant extract (mg); m_o is the weight of rutin in the solution (mg).

Determination of total flavonol content

Total flavonols in the plant extracts were determined using the method of Kumaran and Karunakaran (2007).[27] Prepared different concentrations (100-1000 µg/ml) of root and inflorescences extract and standards (rutin) then 2.0 ml of $AlCl_3$ and 3.0 ml of sodium acetate solution were added. Incubation of 2.5 h at 20°C was provided and absorbance was taken at 440 nm. Total content of flavonol in rutin equivalents (REs) was calculated by the following formula:

$$C = c. V/m$$

Where C is the total content of flavonol compounds, mg/g plant extract (REs); c is the concentration of rutin solution, established from the calibration curve (mg/ml); V is the volume of extract (ml); m is the weight of pure plant extract (g).

Determination of total proanthocynidin content

Proanthocynidin is commonly determined by the method of Sun *et al.,* (1998).[28] Prepared different concentrations (100-1000 µg/ml) of extracts and standard rutin. Then added 3 ml of vanillin-methanol solution and 1.5 ml of HCl and incubated at room temperature for 15 min. Then absorbance was taken at 500 nm. Total content of proanthocynidin, in REs was calculated by the following formula:

$$C = c. V/m$$

Where C is the total content of proanthocynidin compounds, mg/g plant extract (REs); c is the concentration of rutin established from the calibration curve (mg/ml); V is the volume of extract (ml); m is the weight of pure plant extract (g).

Determination of total tannins

Tannin content was determined by vanillin hydrochloride method of Sadashivam and Manickam (1996).[29] Prepared different concentrations (100-1000 µg/ml) of extracts and standard-gallic acid. Then added 5 ml of vanillin hydrochloride reagent and allowed to stand for 20 min. The absorbance was measured at 500 nm. Total content of tannin in GAE was calculated by the following formula:

$$C = c. V/m'$$

Where C is the total content of tannin compounds, mg/g plant extract (GAE); c is the concentration of rutin established from the calibration curve (mg/ml); V is the volume of extract (ml); m is the weight of pure plant extract (g).

Quantitative antioxidant assays

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

The free radical DPPH is reduced to the corresponding hydrazine when it reacts with the hydrogen donors.[30] Prepared different concentrations (100-1000 µg/ml) of extracts and standard-ascorbic acid. Added 5 mi of DPPH stock solution in each test tube and incubated for 30 min

at room temperature. Absorbance was measured at 517 nm against blank (only methanol). Scavenging activity was expressed as the percentage inhibition, calculated using the following formula:

$$\% \text{ inhibition activity} = \text{control absorbance} - \text{sample absorbance} \times 100/\text{control absorbance}.$$

Determination of TAC

Total antioxidant activity was determined by Prieto *et al.,* (1999) method.[31] Prepared different concentrations (100-1000 µg/ml) of extracts and standard-gallic acid. Reagent mixture of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate (1 ml) was added in each test tube. Tubes were capped and incubated in thermal block at 95°C for 90 min and cooled at room temperature. Absorbance was measured at 695 nm against blank. The TAC was expressed as the number of equivalents of gallic acid (GAE) and was calculated by the following formula:

$$A = (c \times V)/m$$

Where A is the total content of antioxidant compounds, mg/g plant extract, in gallic acid; c represents the concentration of gallic acid established from the calibration curve, mg/ml; V is the volume of extract in milliliter; m is the weight of pure plant extract (g).

FRAP (ferric reducing antioxidant power) assay

A modified method of Benzie and Strain (1996)[32] was adopted for the FRAP assay. Prepared different concentrations of extract (100-1000 µg) and standard-$FeSO_4$. Added 2.85 ml of the working solution of FRAP. Incubated for 30 min in the dark condition and the absorbance was taken at 593 nm. Increased absorbance of the reaction mixture indicates increase in FRAP of the extract.

RESULTS AND DISCUSSION

In vitro physicochemical quantitative assays

Total phenol content

The results of the phenol content of various sequential extracts of *A. aspera* roots and inflorescences are given in Tables 1 and 2. The total phenol contents of plant extracts was calculated from regression equation of calibration curve ($y = 0.062\times + 0.151$, $R^2 = 0.988$) and was expressed as GAE. The phenol content of the studied plant extracts was varied from 0.39 to 400 mg/50 g of the dry weight of plant powder. Maximum phenol content was observed in ethyl acetate extract of root (400 mg/50 g) and inflorescences (291.67 mg/50 g). Phenolics are monomeric components of the polymeric polyphenols that make up plant tissues including lignin, melanin, and tannins. Plants have considerable heath benefits due to these antioxidants.[33-35] Plant phenol acting as primary antioxidants or free radical terminators because of their scavenging ability.[36] The high content of phenol compounds found in the extracts may be a contributing factor towards antioxidant activity because the phenolic compounds are known to have direct antioxidant property due to the presence of hydroxyl groups that can function as hydrogen donor.[37,38] Several studies have also revealed that the phenolic content in the plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.[39-41] The antioxidant activity of *A. aspera* is probably due to its phenol content. High phenolic content increases the antioxidant activity and there is a linear correlation between phenol content and antioxidant activity.[42,43]

Table 1: Total phenol, flavonoid, and flavonol content of different extracts of *A. aspera* roots

Extracts	Total phenol (mg/50 g plant extract (GAE))	Total flavonoid (mg/50 g plant extract (REs))	Total flavonol (mg/50 g plant extract (REs))
PEAA	88.10±10.02	0.054±0.011	273.81±21.08
BEAA	150.00±5.00	0.035±0.012	38.24±1.98
CEAA	14.44±2.22	0.021±0.002	2.50±1.25
EAEAA	400±57.00	0.101±0.008	362.5±1.2
EEAA	240.7±8.19	0.011±0.005	1.84±1.01
AEAA	16.37±3.06	0.009±0.008	14.33±2.03

The values are the average of three determinations and are expressed as mean±SD, GAE: Gallic acid equivalent, REs: Rutin equivalents, PEAA: Petroleum ether extract of *A. aspera*, BEAA: Benzene extract of *A. aspera*, CEAA: Chloroform extract of *A. aspera*, EAEAA: Ethyl acetate extract of *A. aspera*, EEAA: Ethanolic extract of *A. aspera*, AEAA: Aqueous extract of *A. aspera*, SD: Standard deviation

Table 2: Total phenol, flavonoid, and flavonol content of different extracts of *A. aspera* inflorescences

Extracts	Total phenol (mg/50 g plant extract (GAE))	Total flavonoid (mg/50 g plant extract (REs))	Total flavonol (mg/50 g plant extract (REs))
PEAA	3.96±0.05	0.031±0.005	5.18±0.03
BEAA	16.66±2.22	0.150±0.02	1023.81±2.01
CEAA	21.97±0.92	0.102±0.006	30.30±0.9
EAEAA	291.67±0.67	0.129±0.01	87.50±4.4
EEAA	0.39±0.04	0.012±0.011	8.41±0.04
AEAA	4.37±0.02	0.002±0.001	6.58±1.16

The values are the average of three determinations and are expressed as mean±SD, GAE: Gallic acid equivalent, REs: Rutin equivalents, PEAA: Petroleum ether extract of *A. aspera*, BEAA: Benzene extract of *A. aspera*, CEAA: Chloroform extract of *A. aspera*, EAEAA: Ethyl acetate extract of *A. aspera*, EEAA: Ethanolic extract of *A. aspera*, AEAA: Aqueous extract of *A. aspera*, SD: Standard deviation

Total flavonoid content

Total flavonoid contents of various sequential extracts are summarized in Tables 1 and 2. Total flavonoid content of the studied plant extracts was varied from 0.002 to 0.15 mg/g of the dry weight. The flavonoid content of different fractions of *A. aspera* root is in the following order: EAEAA (ethyl acetate extract of *A. aspera*) < PEAA (petroleum ether extract of *A. aspera*) < BEAA (benzene extract of *A. aspera*) < CEAA (chloroform extract of *A. aspera*) < EEAA (ethanolic extract of *A. aspera*) < AEAA (aqueous extract of *A. aspera*.), and different fractions of *A. aspera* inflorescences possesses flavonoid content is in following order: BEAA < EAEAA < CEAA

<PEAA <EEAA <AEAA. Benzene extract of inflorescences showed maximum flavonoid content. Flavonoids are natural phenols[44,45] and low molecular weight substances that are usually subdivided into flavonols, flavones, anthocyanidins, and chalcones. The hydroxylation and alkoxylation pattern of the A and B rings of these compounds vary extensively and are of great importance in determining their activity as antioxidants. These compounds possess various biological activities such as anticarcinogenic, anti-inflammatory, and anti-atherosclerotic activities. These biological activities might be related to their antioxidant activity. The antioxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals.[46,47]

Total flavonol content

Total flavonol content of various sequential extracts of *A. aspera* root and inflorescences are summarized in Tables 1 and 2, respectively. The total flavonol contents of plant extracts were calculated from regression equation of calibration curve ($y = 0.018\times - 0.019$, $R^2 = 0.882$) and was expressed as REs. The flavonol content of the studied plant extracts is varied from 1.84 to 1023.81 mg/50 g of the dry weight. Maximum flavonol content was observed in petroleum ether extract of root (273.81 mg/50 g) and benzene extract of inflorescences (1023.81mg/50 g). Plant flavonols are the most important natural compounds that act as potent free radical scavengers and metal chelators[48] because flavonols contain a hydroxyl group (OH^-) in the 3- position of the flavonoid skeleton, which allows them to chelate metals, inhibiting the formation of free radicals and ROS accumulation.[49] Flavonol content is responsible for the excellent antioxidant potential of *A. aspera*.

Total proanthocynidin content

Total proanthocynidin content of various sequential extracts of *A. aspera* roots and inflorescences is summarized in Tables 3 and 4, respectively. The total proanthocynidin contents of plant extracts were calculated from regression equation of calibration curve ($y = 0.005\times - 0.005$, $R^2 = 0.951$) and expressed as REs. The proanthocynidin content of the studied plant extracts was varied from 0.693 to 600 mg/50 g of the dry weight. Maximum proanthocynidin content was observed in ethyl acetate extract of root (600 mg/50 g) and inflorescences (112.5 mg/50 g). Proanthocynidins are a type of bioflavonoids that have been shown to have potent antioxidant activity.[50] These are condensed tannins (proanthocyanidin) belonging to a health-promoting component found in plant-derived foods and beverages and possesses anticarcinogenic and antimutagenic potentials as well as antimicrobial properties.[51-53]

Total tannin content

Total tannin content of various sequential extracts was given in Tables 3 and 4. The total tannin contents of plant extracts were calculated from regression equation of calibration curve ($y = 0.173\times - 0.208$, $R^2 = 0.988$) and was expressed as REs. The tannin content of the studied plant extracts was varied from 0.497 to 1262.50 mg/50 g of the dry weight. The tannin content of different fractions of *A. aspera* roots is in the following order: EAEAA < PEAA < CEAA < BEAA < EEAA < AEAA, and different fractions of *A. aspera* inflorescences possess flavonoid content is in following order: EAEAA < BEAA < CEAA < PEAA < EEAA < AEAA. Tannins are polyphenols that are found to prevent urinary tract infection by preventing bacteria from adhering to the walls. Combination of tannin plus anthocyanins can breakdown oxidized cholesterol in the bloodstream and in atherosclerotic plaques. They also possess anti-nutritional effects, following their ability to reduce palatability and digestibility of feedstuff.[54]

Quantitative antioxidant assays

TAC

TAC is shown in Table 5. It is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. TAC of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity (TAC). TAC mainly concentrates on the thermodynamic conversion and measures the number of electrons or radicals donated or quenched by a given antioxidant molecules and measures the

Table 3: Total proanthocynidins and tannins content in different sequential extracts of *A. aspera* roots

Extracts	Total proanthocynidins (mg/50 g plant extract (REs))	Total tannin (mg/50 g plant extract (REs))
PEAA	135.71±20.02	92.24±11.03
BEAA	2.35±0.2	26.76±5.05
CEAA	152.78±30.03	80.42±11.1
EAEAA	600.00±25.00	1262.50±10.5
EEAA	3.89±2.01	26.53±3.03
AEAA	5.85±1.05	6.43±3.02

The values are the average of three determinations and are expressed as mean±SD, GAE: Gallic acid equivalent, REs: Rutin equivalents, PEAA: Petroleum ether extract of *A. aspera*, BEAA: Benzene extract of *A. aspera*, CEAA: Chloroform extract of *A. aspera*, EAEAA: Ethyl acetate extract of *A. aspera*, EEAA: Ethanolic extract of *A. aspera*, AEAA: Aqueous extract of *A. aspera*, SD: Standard deviation

Table 4: Total proanthocynidins and tannins content in different sequential extracts of *A. aspera* inflorescences

Extracts	Total proanthocynidins (mg/50 g plant extract (REs))	Total tannin (mg/50 g plant extract (REs))
PEAA	0.693±0.102	8.37±2.04
BEAA	74.168±4.002	18.06±2.97
CEAA	101.52±3.98	17.23±1.1
EAEAA	112.5±0.60	58.33±0.98
EEAA	4.28±1.04	2.941±0.151
AEAA	3.79±2.02	0.497±0.106

The values are the average of three determinations and are expressed as mean±SD, GAE: Gallic acid equivalent, REs: Rutin equivalents, PEAA: Petroleum ether extract of *A. aspera*, BEAA: Benzene extract of *A. aspera*, CEAA: Chloroform extract of *A. aspera*, EAEAA: Ethyl acetate extract of *A. aspera*, EEAA: Ethanolic extract of *A. aspera*, AEAA: Aqueous extract of *A. aspera*, SD: Standard deviation

Table 5: TAC (mg/g plant extract) of different fractions of the roots and inflorescences of *A. aspera*

Extracts	Roots	Inflorescences
PEAA	371.67±0.015	364.53±0.012
BEAA	396.22±0.029	373.44±0.015
CEAA	417.84±0.007	410.64±0.043
EAEAA	388.81±0.012	393.23±0.049
EEAA	374.26±0.015	426.14±0.021
AEAA	384.25±0.046	373.10±0.033

The values are the average of three determinations and are expressed as mean±SD, GAE: Gallic acid equivalent, REs: Rutin equivalents, PEAA: Petroleum ether extract of *A. aspera*, BEAA: Benzene extract of *A. aspera*, CEAA: Chloroform extract of *A. aspera*, EAEAA: Ethyl acetate extract of *A. aspera*, EEAA: Ethanolic extract of *A. aspera*, AEAA: Aqueous extract of *A. aspera*, SD: Standard deviation, TAC: Total content of antioxidant compounds

capacity of biological samples under defined conditions.[55] Maximum antioxidant capacity was observed in ethanolic extract (426.14 mg/g) of inflorescences and chloroform extract (417.84 mg/g) of roots. This study reveals that the antioxidant activity of the extract exhibits increasing trend with the increasing concentration of the plant extract. Observed results revealed that all extracts were found to have higher activity as compared with the standard (gallic acid) used for this study. The results indicate a concentration-dependent TAC. It means that the extracts of *A. aspera* root and inflorescences contain as much quantity of antioxidants compounds as equivalents of ascorbic acid to effectively reduce the oxidant in the reaction matrix.

DPPH radical scavenging activity

To evaluate free radical scavenging activity of antioxidants, DPPH is usually used.[27,56] DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.[27,50,57] The extracts were able to reduce the stable radical DPPH to the yellow-colored di-phenyl-picryl-hydrazine. The scavenging effect of different fractions of *A. aspera* root and standard (BHT) with the DPPH radical activity is in the following order: STD (96.67%) > EEAA (92.75%) > BEAA (79.22%) > CEAA (63.43%) > AEAA (43.63%) > PEAA (39.31%) > EAEAA (35.10%), respectively, at the dose of 1 mg/ml, and effect of different fractions of *A. aspera* inflorescences and standard is in following order: STD (96.67%) > CEAA (84.80%) > BEAA (80.59%) > PEAA (71.57%) > EAEAA (68.92%) > EEAA (52.35%) > AEAA (45.59%), respectively, at the dose of 1 mg/ml. Maximum DPPH scavenging activity was found in ethanolic extract of (root) and chloroform extract (inflorescences) of *A. aspera*. The experimental data of *A. aspera* revealed that all these extracts likely to have the effect of scavenging free radical activity at different concentration of extracts. From Figures 1 and 2, we observed dose-dependent relationship in the DPPH radical scavenging activity, the activity increased as the concentration increased for both parts of *A. aspera*. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines reduce and decolorized DPPH by their hydrogen donating

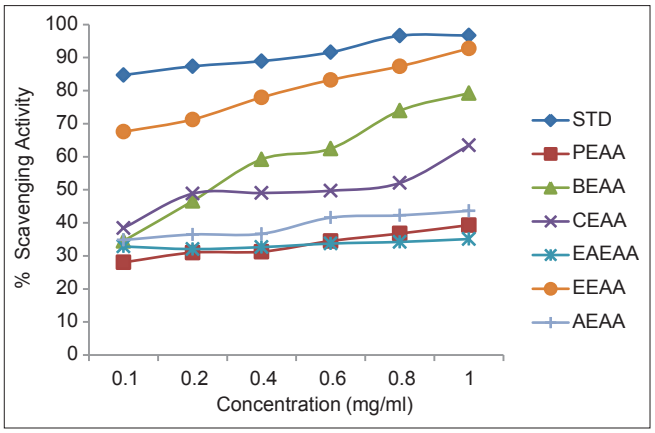

Figure 1: DPPH-free radical scavenging activity of various sequential extracts of root of *A. aspera* DPPH = 1,1-diphenyl-2-picrylhydrazyl

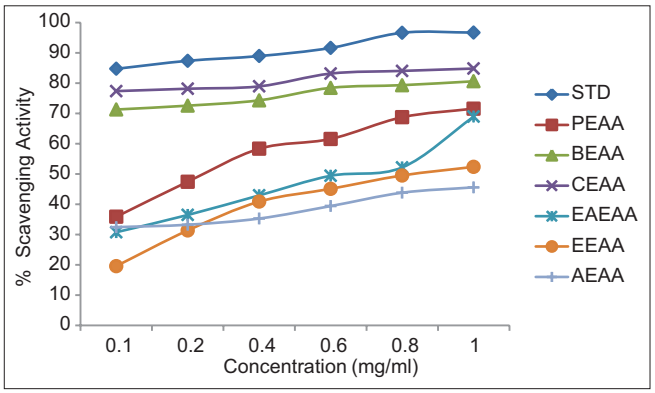

Figure 2: DPPH-free radical scavenging activity of various sequential extracts of inflorescences of *A. aspera* DPPH = 1,1-diphenyl-2-picrylhydrazyl

ability.[27,58,59] Therefore, the phenol compounds of *A. aspera* extracts may probably be involved in their antiradical activity.

FRAP assay

The ability of plant extract to reduce ferric ions was determined in FRAP assay. The change in absorbance at 593 nm owing to the formation of blue-colored Fe^{+2} tri-pyridyltriiazine (TPTZ) compound from the colorless oxidized Fe^{+3} form by the action of electron donating antioxidants. The FRAP values of extract were found to be significantly higher as compared with the standards ($FeSO_4$). FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present, thus it can be reported that extract of *A. aspera* may act as free radical scavenger, capable of transforming reactive free radical species into stable non-radical products. Antioxidant activity increased proportionally with the polyphenol content. In comparison with standard, plant extracts gave satisfactory result. From Figures 3 and 4, we observed dose-dependent relationship in the reducing antioxidant power, the power increased as the concentration increased for both parts (root and inflorescences) of *A. aspera*. Phenol compounds have redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The redox

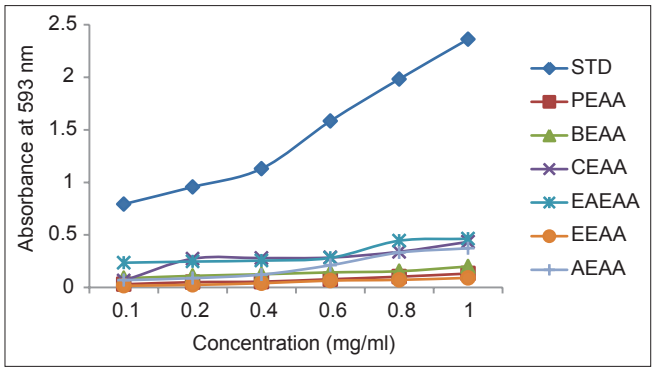

Figure 3: FRAP assay of different sequential extracts of root of *A. aspera* FRAP = ferric reducing antioxidant power

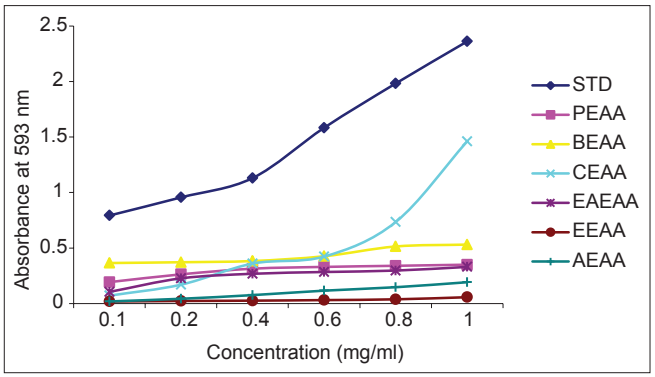

Figure 4: FRAP assay of different sequential extracts of inflorescences of *A. aspera* FRAP = ferric reducing antioxidant power

potential of phenol compounds played an important role in determining the antioxidant potential.[60]

CONCLUSION

From the observations it can be concluded that the roots and inflorescences of *A. aspera* are the good sources of Antioxidant molecules and these antioxidants could be useful in treating the disease associated with oxidative stress. Thus *A. aspera* could be used to manufacture drug for the treatment of various human aliments.

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REFERENCES

1. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 2004;266:37-56.
2. Maramatsu T, Hiramatsu H, Okumura J. Induction of ovalbumin mRNA by ascorbic acid in primary cultures of tubular gland cells of the chicken oviduct. Comp Biochem Physiol B Biochem Mol Biol 1995;112:209-16.
3. Das D, Bandyopadhyay D, Bhattacharjee M, Banerjee RK. Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. Free Radic Biol Med 1997;23:8-18.
4. Beaulah AG, Sadiq MA, Santhi JR. Antioxidant and antibacterial activity of *Achyranthes aspera*: An *in vitro* study. Sch Res Libr 2011;2:662-70.
5. Maxwell SR. Prospects for the use of antioxidant therapies. Drugs 1995;49:345-61.
6. Samarakon SM, Chandola HM, Shukla VJ. Evaluation of antioxidant potential of *Amalakayas Rasayana*: A polyherbal Ayurvedic formulation. Int J Ayurveda Res 2011;2:23-8.
7. Sharma V, Janmeda P, Paliwal R, Sharma S. Antihepatotoxic activity of *Euphorbia neriifolia* extract against N-nitrosodiethylamine-induced hepatocarcinogenesis in mice. Zhong Xi Yi Jie He Xue Bao 2012;10:1303-9.
8. Sharma V, Chaudhary U, Singh R, Agarwal A. *Achyranthes aspera*: Phytochemical estimation. Am J Pharmtech Res 2013;3:242-51.
9. Sharma V, Agarwal A, Chaudhary U, Singh M. Phytochemical investigation of various extracts of leaves and stems of *Achyranthes aspera* Linn. Int J Pharm Pharm Sci 2013;5:317-20.
10. Kumar RU, Begum HV. Antimicrobial studies of some selected medicinal plants. Anc Sci Life 2002;21:230-9.
11. Kumar SS, Perumal P, Boopathy D, Mukherjee PK, Suresh B. Comparative microbiological activities of ethanolic extracts of roots and aerial parts of *Achyranthes aspera* Linn. Anc Sci Life 2003;22:140-5.
12. Kaur M, Thakur Y, Rana RC. Antimicrobial properties of *Achyranthes aspera*. Anc Sci Life 2005;24:168-73.
13. Sharma V, Singh R, Chaudhary U. Curative efficacy of *Achyranthes aspera* against various pathogenic microbial strains. Int J Drug Dev Res 2013;5:392-401.
14. Sharma V, Singh R, Paliwal R, Chaudhary U, Agarwal A. MIC values of inflorescences and leaves extracts of *Achyranthes aspera* against usual pathogenic bacterial strains. Asian J Pharm Clin Res 2013;6:185-7.
15. Akhtar MS, Iqbal J. Evaluation of the hypoglycaemic effect of *Achyranthes aspera* in normal and alloxan-diabetic rabbits. J Ethnopharmacol 1991;31:49-57.
16. Khanna AK, Chander R, Singh C, Srivastava AK, Kapoor NK. Hypolipidemic activity of *Achyranthes aspera* Linn in normal and triton induced hyperlipemic rats. Indian J Exp Biol 1992;30:128-30.
17. Talukder FZ, Khan KA, Uddin R, Jahan N, Alam MA. *In vitro* free radical scavenging and anti-hyperglycemic activities of *Achyranthes aspera* extract in alloxan-induced diabetic mice. Drug Discov Ther 2012;6:298-305.
18. Chakraborty A, Brantner A, Mukainaka T, Nobukuni Y, Kuchide M, Konoshima T, *et al.* Cancer chemopreventive activity of *Achyranthes aspera* leaves on Epstein-Barr virus activation and two-stage mouse skin carcinogenesis. Cancer Lett 2002;177:1-5.
19. Gokhale AB, Damre AS, Kulkami KR, Saraf MN. Preliminary evaluation of anti-inflammatory and anti-arthritic activity of *S. lappa, A. speciosa* and *A. aspera*. Phytomedicine 2002;9:433-7.
20. Gupta SS, Bhagwat AW, Ram AK. Cardiac stimulant activity of the saponin of *Achyranthes aspera* (Linn). Indian J Med Res 1972;60:462-71.
21. Anantha KC, Siva RC, Manoha RA. Hepatoprotective effect of biherbal ethanolic extract against paracetamol-induced hepatic damage in albino rats. J Ayurveda Integr Med 2012;3:198-203.
22. Rao VY, Chakrabarti R. Enhanced anti-proteases in *Labeo rohita* fed with diet containing herbal ingredients. Indian J Clin Biochem 2004;19:132-4.
23. Zahir AA, Rahuman AA, Kamaraj C, Bagavan A, Elango G, Sangaran A, *et al*. Laboratory determination of efficacy of indigenous plant extracts for parasites control. Parasitol Res 2009;105:453-61.
24. Bagavan A, Rahuman AA, Kamaraj C, Geetha K. Larvicidal activity of saponin from *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res 2008;103:223-9.
25. Folin O, Ciocalteu V. Tyrosin and tryptophan determination in proteins. J Biol Chem 1927;73:627-50.
26. Ordon EA, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) swart extracts. Food Chem 2006;97:452-8.
27. Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT-Food Sci Tech 2007;40:344-52.
28. Sun JS, Tsuang YH, Chen IJ, Huang WC, Hang YS, Lu FJ. An ultra-weak

chemiluminescence study on oxidative stress in rabbits following acute thermal injury. Burns 1998;24:225-31.
29. Sadashivam S, Manickam A. Biochemical methods. 2nd ed. 1996. p. 121-4.
30. Tagashira M, Ohtake Y. A new antioxidative 1,3-benzodioxole from *Melissa officinalis*. Planta Med 1998;64:555-8.
31. Prieto P, Pineda M, Aguilar M. Spectrophotometeric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem1999;269:337-41.
32. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as measure of "antioxidant power": The FRAP assay. Anal Biochem 1996;239:70-6.
33. Sarni-Manchado P, Le Roux E, Le Guerneve C, Lozano Y, Cheynier V. Phenolic composition of litchi fruit pericarp. J Agric Food Chem 2000;48:5995-6002.
34. Gorinstein S, Caspi A, Libman I, Lerner HT, Huang D, Lentowich H, *et al*. Red grapefruit positively influences serum triglyceride level in patients suffering from coronary atherosclerosis: Studies *in vitro* and in humans. J Agric Food Chem 2006;54:1887-92.
35. Park YS, Jung ST, Kang SG, Drzewiecki J, Namiesnik J, Haruenkit R, *et al*. *In vitro* studies of polyphenols, antioxidants and other dietary indices in kiwifruit (*Actinidia deliciosa*). Int J Food Sci Nutr 2006;57:107-22.
36. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behaviour of flavonoids: Structure activity relationships. Free Radic Biol Med 1997;22:749-60.
37. Duh PD, Tu YY, Yen GC. Antioxidant activity of the aqueous extract of harn jyur (*Chrysanthemum morifolium Ramat*). Lebenson Wiss Technol 1999;32:269-77.
38. Dreosti IE. Antioxidant polyphenols in tea, cocoa and wine. Nutrition 2000;16:692-4.
39. Moller JK, Madsen HL, Aaltonen T, Skibsted LH. Dittany (*Origanum dictamnus*) as a source of water-extractable antioxidants. Food Chem 1999;64:215-9.
40. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. J Agric Food Chem 2001;49:3420-4.
41. Adedapo AA, Jimoh FO, Koduru S, Masika PJ, Afolayan AJ. Evaluation of the medicinal potentials of the methanol extracts of the leaves and stems of *Halleria lucida*. Bioresour Technol 2008;99:4158-63.
42. Geldof N, Engeseth NJ. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples. J Agric Food Chem 2002;50:3050-5.
43. Kumaran A, Karunakaran RJ. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. Food Chem 2006;97:109-14.
44. Miliauskas G, Venskutonis PR, Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004;85:231-7.
45. Agarwal PK. Carbon-13 NMR of Flavonoids. New York: Elsevier Science Publishers, Amsterdam; 1989.
46. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: A review. Crit Rev Food Sci Nutr 1998;38:421-64.
47. Wei SD, Zhou HC, Lin YM. Antioxidant activities of extract and fractions from the hypocotyls of the mangrove plant Kandelia candel. Int J Mol Sci 2010;11:4080-93.
48. Tanaka M, Kuie CW, Nagashima Y, Taguchi T. Applications of antioxidative maillard reaction products from histidine and glucose to sardine products. Nippon Suisan Gakk 1988;54:1409-14.
49. Agati G, Stefano G, Biricolti S, Tattini M. Mesophyll distribution of antioxidant Flavonoids in *Ligustrum vulgare* leaves under contrasting sunlight irradiance. Ann Bot 2009;104:853-61.
50. Pracheta, Sharma V, Paliwal R, Sharma S. *In vitro* free radical scavenging and antioxidant potential of ethanolic extract of *Euphorbia nerifolia* Linn. Int J Pharm Pharm Sci 2011;3:238-42.
51. Chung KT, Stevens SE, Lin WF. Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. Lett Appl Microbiol 1993;17:29-35.
52. Katiyar SK, Mukhtar H. Tea antioxidants in cancer chemoprevention. J Cell Biochem Suppl 1997;27:59-67.
53. Amarowicz R, Pegg RB, Bautista DA. Antibacterial activity of green tea polyphenols against *Escherichia coli K12*. Nahrung 2000;44:60-2.
54. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian Medicinal plants. 2nd OAU/STRC Inter-African Symposium on traditional Phamacopoeia and African Medicinal; 1979. p. 216-20.
55. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: Critical view and experimental data. Free Radic Biol Med 2000;29:1106-14.
56. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nut 1986;44:307-15.
57. Soares JR, Dinis TC, Cunha AP, Almeida LM. Antioxidant activities of some extracts of *Thymus zygis*. Free Radic Res 1997;26:469-78.
58. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958;181:1199-2000.
59. Yokogawa J, Imanishi K, Tsujimoto M, Koyama K. In Japanese-German workshop on high-energy physics. In: Becker W, Itoh M, editor. (MPE Rep. 270; Garching: MPE), 151 ADS; 1998.
60. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci 1997;4:304-9.

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