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 proanthocyanidin 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.[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. 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:

\[ C = \frac{c \cdot V}{m} \]

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 volume of extract (ml); \( m \) is the weight of pure plant extract (g).

Determination of total flavonoid content
Total flavonols in the plant extracts were determined using the method of Kumaran and Karunakaran (2007). Prepared different concentrations (100-1000 µg/ml) of root and inflorescences extract and standards (rutin) then 2.0 ml of AlCl₃ 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:

\[ C = \frac{c \cdot V}{m} \]

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 proanthocyanidin content
Proanthocyanidin is commonly determined by the method of Sun et al., (1998). 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 proanthocyanidin, in REs was calculated by the following formula:

\[ C = \frac{c \cdot V}{m} \]

Where \( C \) is the total content of proanthocyanidin 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). 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:

\[ C = \frac{c \cdot V}{m} \]

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. Prepared different concentrations (100-1000 µg/ml) of extracts and standard-ascorbic acid. Added 5 ml of DPPH stock solution in each ascorbic acid. Added 5 ml of DPPH stock solution in each
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:

\[
\% \text{ inhibition activity} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100
\]

**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:

\[
A = \frac{(c \times V)}{m}
\]

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)\[^{[25]}\] 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 = 0.062x + 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 donors.\[^{[37,38]}\] 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.\[^{[42,43]}\]

**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: EAAE (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*) < AEA (aqueous extract of *A. aspera*)., and different fractions of *A. aspera* inflorescences possesses flavonoid content is in following order: BEAA < EAAE < CEAA

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

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total phenol (mg/50 g plant extract GAE)</th>
<th>Total flavonoid (mg/50 g plant extract REs)</th>
<th>Total flavonol (mg/50 g plant extract REs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEAA</td>
<td>88.10±10.02</td>
<td>0.054±0.011</td>
<td>273.81±21.08</td>
</tr>
<tr>
<td>BEAA</td>
<td>150.00±5.00</td>
<td>0.035±0.012</td>
<td>38.24±1.98</td>
</tr>
<tr>
<td>CEAA</td>
<td>14.4±2.22</td>
<td>0.021±0.002</td>
<td>2.50±1.25</td>
</tr>
<tr>
<td>EAAE</td>
<td>400±57.00</td>
<td>0.101±0.008</td>
<td>362.5±1.2</td>
</tr>
<tr>
<td>EEA</td>
<td>240.7±8.19</td>
<td>0.011±0.005</td>
<td>1.84±1.01</td>
</tr>
<tr>
<td>AEA</td>
<td>16.37±3.06</td>
<td>0.009±0.008</td>
<td>14.33±2.03</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total phenol (mg/50 g plant extract GAE)</th>
<th>Total flavonoid (mg/50 g plant extract REs)</th>
<th>Total flavonol (mg/50 g plant extract REs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEAA</td>
<td>3.96±0.05</td>
<td>0.031±0.005</td>
<td>5.18±0.03</td>
</tr>
<tr>
<td>BEAA</td>
<td>16.66±2.22</td>
<td>0.150±0.02</td>
<td>1023.81±21.08</td>
</tr>
<tr>
<td>CEAA</td>
<td>21.97±0.92</td>
<td>0.102±0.006</td>
<td>30.30±0.9</td>
</tr>
<tr>
<td>EAAE</td>
<td>291.67±6.70</td>
<td>0.129±0.01</td>
<td>87.50±4.4</td>
</tr>
<tr>
<td>EEA</td>
<td>0.39±0.04</td>
<td>0.012±0.011</td>
<td>8.41±0.04</td>
</tr>
<tr>
<td>AEA</td>
<td>4.37±0.02</td>
<td>0.002±0.001</td>
<td>6.58±1.16</td>
</tr>
</tbody>
</table>

}\[^{[31]}\] 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*, AEA: Ethyl acetate extract of *A. aspera*, EEA: Ethanol extract of *A. aspera*, AEA: Aqueous extract of *A. aspera*, SD: Standard deviation

\[^{[25]}\] 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*, AEA: Ethyl acetate extract of *A. aspera*, EEA: Ethanol extract of *A. aspera*, AEA: Aqueous extract of *A. aspera*, SD: Standard deviation
Total flavonol content

Total flavonol content of various sequential extracts of *A. aspera* root and inflorescences is summarized in Tables 1 and 2, respectively. The total flavonol contents of plant extracts were calculated from regression equation of calibration curve (\(y = 0.018x - 0.019, R^2 = 0.882\)) 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 (1023.81 mg/50 g). Plant flavonols are the most important natural compounds that act as potent free radical scavengers and metal chelators 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. Flavonol content is responsible for the excellent antioxidant potential of *A. aspera*.

Total proanthocyanidin content

Total proanthocyanidin content of various sequential extracts of *A. aspera* roots and inflorescences is summarized in Tables 3 and 4, respectively. The total proanthocyanidin contents of plant extracts were calculated from regression equation of calibration curve (\(y = 0.005x - 0.005, R^2 = 0.951\)) and was expressed as REs. The proanthocyanidin content of the studied plant extracts was varied from 0.693 to 600 mg/50 g of the dry weight. Maximum proanthocyanidin content was observed in ethyl acetate extract of root (600 mg/50 g) and inflorescences (112.5 mg/50 g). Proanthocyanidins are a type of bioflavonoids that have been shown to have potent antioxidant activity. 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.

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 (\(y = 0.173x - 0.208, R^2 = 0.988\)) 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 < AEA and different fractions of *A. aspera* inflorescences possess flavonoid content is in following order: EAEAA < BEAA < CEAA < PEA < EEAA < AEA. 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.

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 antioxidant activity of the extracts.

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

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total proanthocyanidins (mg/50 g plant extract (REs))</th>
<th>Total tannin (mg/50 g plant extract (REs))</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEAA</td>
<td>135.71±20.02</td>
<td>92.24±11.03</td>
</tr>
<tr>
<td>BEAA</td>
<td>2.35±0.2</td>
<td>26.76±5.05</td>
</tr>
<tr>
<td>CEAA</td>
<td>152.78±30.03</td>
<td>80.42±11.1</td>
</tr>
<tr>
<td>EAEAA</td>
<td>600.00±25.00</td>
<td>1262.50±10.5</td>
</tr>
<tr>
<td>EEA</td>
<td>3.89±2.01</td>
<td>26.53±3.03</td>
</tr>
<tr>
<td>AEA</td>
<td>5.85±1.05</td>
<td>6.43±3.02</td>
</tr>
</tbody>
</table>

The values are the average of three determinations and are expressed as mean±SD.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total proanthocyanidins (mg/50 g plant extract (REs))</th>
<th>Total tannin (mg/50 g plant extract (REs))</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEAA</td>
<td>0.69±0.102</td>
<td>8.37±2.04</td>
</tr>
<tr>
<td>BEAA</td>
<td>74.16±4.002</td>
<td>18.06±2.97</td>
</tr>
<tr>
<td>CEAA</td>
<td>101.52±3.98</td>
<td>17.23±1.1</td>
</tr>
<tr>
<td>EAEAA</td>
<td>112.5±0.60</td>
<td>58.33±0.98</td>
</tr>
<tr>
<td>EEA</td>
<td>4.28±1.04</td>
<td>2.94±0.15</td>
</tr>
<tr>
<td>AEA</td>
<td>3.79±2.02</td>
<td>0.497±0.106</td>
</tr>
</tbody>
</table>

The values are the average of three determinations and are expressed as mean±SD.

capacity of biological samples under defined conditions.\textsuperscript{[53]} 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 \textit{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.\textsuperscript{[27,56]} DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.\textsuperscript{[27,50,57]} The extracts were able to reduce the stable radical DPPH to the yellow-colored di-pierylhydrazone. The scavenging effect of different fractions of \textit{A. aspera} root and standard (BHT) with the DPPH radical activity is in the following order: STD (96.67%) > EEA (92.75%) > BEAA (79.22%) > CEAA (63.43%) > AEAA (43.63%) > PEAA (39.31%) > EEAA (35.10%), respectively, at the dose of 1 mg/ml, and effect of different fractions of \textit{A. aspera} inflorescences and standard is in following order: STD (96.67%) > CEAA (84.80%) > BEAA (80.59%) > PEAA (71.57%) > EEA (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 \textit{A. aspera}.

The experimental data of \textit{A. aspera} revealed that all these extracts likely 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 \textit{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 ability.\textsuperscript{[27,58,59]} Therefore, the phenol compounds of \textit{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($^{3+}$)-tri-pyridyltriazine (TPTZ) compound from the colorless oxidized Fe($^{2+}$) 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 \textit{A. aspera} may act as a 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 \textit{A. aspera}. Phenol compounds have redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. The redox

\textbf{Table 5: TAC (mg/g plant extract) of different fractions of the roots and inflorescences of \textit{A. aspera}}

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Roots</th>
<th>Inflorescences</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEAA</td>
<td>371.67±0.015</td>
<td>364.53±0.012</td>
</tr>
<tr>
<td>BEAA</td>
<td>396.22±0.029</td>
<td>373.44±0.015</td>
</tr>
<tr>
<td>CEAA</td>
<td>417.84±0.007</td>
<td>410.64±0.043</td>
</tr>
<tr>
<td>EEA</td>
<td>388.81±0.012</td>
<td>393.23±0.049</td>
</tr>
<tr>
<td>EEA</td>
<td>374.26±0.015</td>
<td>426.14±0.021</td>
</tr>
<tr>
<td>AEAA</td>
<td>384.25±0.046</td>
<td>373.10±0.033</td>
</tr>
</tbody>
</table>

The values are the average of three determinations and are expressed as mean±SD. GAE: Gallic acid equivalent, REs: Rutin equivalents, PEA: Petroleum ether extract of \textit{A. aspera}, BEAA: Benzene extract of \textit{A. aspera}, CEAA: Chloroform extract of \textit{A. aspera}, EEA: Ethyl acetate extract of \textit{A. aspera}, EEAA: Ethanolic extract of \textit{A. aspera}, AEA: Aqueous extract of \textit{A. aspera}, SD: Standard deviation, TAC: Total content of antioxidant compounds.
potential of phenol compounds played an important role in determining the antioxidant potential.\cite{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 ailments.

**ACKNOWLEDGMENTS**

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**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

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