Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND STUDY ON ANTIOXIDANT PROPERTY OF *AMARANTHUS CAUDATUS* LINN.

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ABSTRACT

The present study was carried out for the analysis of dietary antioxidants viz phenolics and flavonoids from the leaves of *A. caudatus* which is an annual herb and a popular leafy vegetable. The antioxidant potency of the species was analysed *in vitro* by DPPH reduction test and by reducing power assay. The phenolic content was 2.98 GAE/g while flavonoid content was found to be 5.19 RUE/g. The *in vitro* antioxidant activities were found to be impressive by both the assay methods. RSA was found to be 41.6mg GAE/g by DPPH test. The IC₅₀ value was found to be 490µl. Moreover preliminary phytochemical analysis showed the presence of saponin, tannin and terpenoid in methanolic extract.

Keyword: phenolic, flavonoid, DPPH, reducing power assay, IC₅₀, antioxidant activity

INTRODUCTION

Dietary antioxidants are gaining increasing importance because of their health protective value, easy availability and biosafety. In contrast to that, synthetic antioxidants are generally used as additive to processed food and cannot be consumed directly. In recent time the harmful effect of synthetic antioxidants like BHT-Butylated hydroxytoluene, BHA-Butylated hydroxyanisole etc are coming to light and so therefore their biosafety aspect is questionable^{1,2}. Dietary antioxidants are generally secondary metabolites found only in plants and a large number of which varies nut, seed, root, leafy vegetables and spices are known to have antioxidants in various amount³. Some of the well characterized dietary antioxidants are phenolics, flavonoid, pigments like anthocyanin, betacyanin, vitamin C, vitamin E etc⁴. Antioxidants primarily function by neutralizing free radical which are routinely generated in course of normal metabolic processes like respiration, other activities like inflammation, infection etc. However our body has a number of antioxidant enzymes as in-built protection device against the damaging free radical. However when production of free radicals exceeds the natural scavenging property they cause oxidative damage to vital biomolecules like DNA, RNA, protein; damage membrane by lipid peroxidation⁵ and tissue. All of these oxidative damage are root cause for a number of health problems like cancer, coronary heart disease, ageing and a variety of degenerative diseases⁶.

There are reports that factors like pollution, cigarette smoke, alcohol, certain drugs, physical and psychological stress can also enhance generation of free radical⁷ which is aggravating human health. Against this backdrop there is an urgency to screen various food plants particularly lesser known plant as readily available and effective dietary antioxidant. Working with four leafy vegetables reported high amount of phenolics and flavonoid^{8,9}. Among the 20 known *in vitro* methods DPPH reduction test is widely used by great majority of the researchers¹⁰.

A. caudatus Linn, (Amaranthaceae) commonly known as Morisa sak in Assamese which is cultivated in kitchen garden. *A. caudatus* is an annual flowering plant whose leaves and stems are edible. They are

used in various recipes. A part from being used as a leafy vegetable; the plant is known to have medicinal value. Traditionally the plant parts of *A. caudatus* are used in the treatment of diseases like jaundice and amoebiasis. It also helps in various kidney related diseases^{11,12}. It has also been reported for activities like antipyretic, antihelminthic^{13,14} and anti-atherosclerotic¹⁵.

MATERIALS AND METHODS

Fresh leaves of *A. caudatus* were collected from natural habitat of Kamrup district, Assam, India. The leaves were washed properly and air-dried to crispiness in the laboratory at room temperature. For identification of the specimen herbarium was prepared and authenticated in the department of Botany, Gauhati University, Assam, India.

Extraction of plant materials

The dried leaves were grinded in a mechanical grinder to powdered form. For extraction 500mg dried sample was homogenized with methanol. The mixture was extracted for 24hrs by constant shaking in a shaker. Thereafter the mixture was centrifuged to obtain the supernatant. The residue was washed twice with methanol and supernatants pooled. The supernatant was used for preliminary phytochemical tests.

Preliminary phytochemical investigation

The preliminary phytochemical investigation was done using standard procedures^{16,17,18,19}

Test for saponin A small quantity of the extract was diluted with 4ml of distilled water in a graduated cylinder. The mixture was shaken manually and then allowed to stand. Formation of froth showed the presence of saponin.

Test for tannin 1ml of filtrate was mixed to 1ml of distilled water and then two drops of ferric chloride solution. A transient greenish to black colour indicates the presence of tannin.

Test for terpenoid (salkowski test) 1ml of the extract was mixed with 2ml of 1% aluminum chloride solution and observed for light yellow colour. It indicated the presence of flavonoid. Subsequently to the solution, dilute NaOH and HCL was added. Discolouration of yellow colour confirmed the presence of terpenoid.

Test for phenol The extract was taken in a test tube and mixed with distilled water and warmed. To this 2ml ferric chloride solution was added and observed for the formation of green or blue color.

Test for flavonoids 0.5g of the dried sample was mixed with 10ml of ethyl acetate and heated in boiling water bath for 1min. The mixture was then filtered. 4ml of the filtrate was shaken with 1ml of 1% aluminum chloride solution. Subsequently 1ml of dilute ammonia solution was added to the mixture. Formation of yellow colour indicated the presence of flavonoids.

Quantification of flavonoid content

Total of the sample was extracted and estimated as per the protocol of Jia *et.al.* 1999. 300mg of finely grinded sample was extracted with 80% methanol for 5hrs in a magnetic stirrer. Thereafter the mixture was centrifuged to obtain the supernatant. The residue was washed twice with 80% methanol and supernatants were pooled. For colour reaction, to the extract 100µl potassium acetate and 100µl aluminum nitrate was added sequentially. The mixture was allowed to stand for 40 minutes at room temperature following which yellow colouration was observed. The absorbance value was taken in a spectrophotometer at 415nm. Quantification was made from standard curve prepared with Rutin.

Quantification of phenolic content

Total phenol was extracted and quantified as per method of Guleria *et.al.* 2011. 500mg finely grounded dried matter was extracted with 80% ethanol. The mixture was constantly shaken at room temperature in a magnetic stirrer for 8hrs. Thereafter the mixture was centrifuged to obtain the supernatant. From the supernatant ethanol was removed by evaporation and to the residual amount distilled water was added to a total volume of 20ml. Colour reaction was carried out through

sequential addition of Folin reagent and 20% sodium carbonate. The solution was warmed in hot water for 1minute. As a consequence of which blue colour was observed. Absorbance was taken in a UV spectrophotometer at 650nm. Quantification was done from standard curve prepared with Gallic acid. The values were expressed as milligram dry matter.

In vitro antioxidant assays

DPPH (2, 2diphenyl-2-picryl hydrazine)

In vitro antioxidant assay was carried out by DPPH reduction test and reducing power assay. For DPPH test methanol if extract was used. For this 200µl of the extract was taken to which 1.8ml methanol was added. To this 2ml DPPH solution (1mM DPPH prepared in methanol) was added. The mixture was incubated in dark for 30minutes at room temperature. The negative control comprise of 200µl methanol processed similarly. The absorbance was taken in a UV spectrophotometer at 517nm. From the reduction in absorbance for the sample the radical scavenging activity i.e. RSA was calculated using the following equation

$$\%RSA = [(A_0 - A_s) / A_0] \times 100$$

RSA (Reducing power assay)

Reducing power assay was carried out as per the protocol of Oyaizu, 1986. Methanolic extract was used for reducing power assay (RSA). For colour reaction, a known volume (200µl) 2.5ml 1% potassium ferricyanide was added and incubated at 50°C for 20 minutes in a water bath. This was followed by addition of 2.5ml distilled water and .5ml ferric chloride. A greenish colour was observed and the absorbance value was quickly taken in a UV spectrophotometer at 700nm. Quantification was made from the standard curve prepared with Gallic acid. Reducing power was expressed as Gallic acid equivalent (GAE mg/g)

IC50

For determining IC50 value, a gradient solution of methanolic extract was prepared from 100µl to 1000µl with an increment of 100µl. The absorbance values were plotted on a graph against corresponding volume of extract. From this IC value was computed.

Table 1: Results of the preliminary phytochemical study

Serial No	Phytochemical	Result
1	Saponin	+ve
2	Tanin	+ve
3	Terpenoid	+ve
4	Phenol	+ve
5	Flavonoid	+ve

Table 2: Results of the total phenolics and flavonoids content evaluated in the studied sample are summarized below

Sample	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg RUE/g)
<i>A. caudatus</i>	2.98	5.19

Figure 1: Image of *A. caudatus* in natural habitat.

Figure 2: Absorption spectra for methanolic extract of *A. caudatus* with gradient dilution to determine IC-50.

RESULTS

The preliminary phytochemical analysis of *A. caudatus* leaf extract is shown in Table 1.

The amount of phenol and flavonoid in the studied sample are summarized in the Table 2.

Dose – dependent scavenging activity of DPPH radical was shown by both the samples. The percentage inhibition of *A. caudatus* leaf extract was 53.615%.

The reducing power in *A. caudatus* leaf extract was found to be 41.6mg GAE/g.

IC₅₀ value was found to be 490µl of the extract.

DISCUSSION

The study revealed that the leaves of *A. caudatus* has significant amount of flavonoid and phenolics which are recognized as potent antioxidant. A number of workers have reported that leafy vegetables as well as medicinal plants are excellent sources of dietary antioxidants. Working with four leafy vegetables reported phenolics in the range of 6.0-18.0 mg/g and flavonoid in the range of 2.5-15.5mg/g. Similar findings were also reported. Therefore the findings of the present study are comparable to that of the earlier findings.

Antioxidant activity is proportionate to the amount of dietary antioxidant present. In the present study using DPPH test, RSA was found to be 53.62% which is quite impressive. Reducing power has been found to be 41.6mg which is comparable to that of RSA by DPPH test. Therefore it appears that in the present study DPPH reduction test and reducing power assay findings complement each other. Therefore it may be opined that *A. caudatus* has considerable amount of anti-oxidant potency which is preliminary to the presence of phenolics & flavonoid as dietary antioxidant.

The IC₅₀ value in the present study has been found to be 490µl of the extract which is complement to the RSA values.

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