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In vitro Antioxidant activity and Phenolic Content of *Croton caudatum*

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Abstract: The antioxidant activities of different concentrations of ethanol extracts of the leaves of Croton caudatum were determined by the four assay techniques i.e. DPPH radical scavenging assay, Reducing power ability, Hydrogen peroxide scavenging assay and thiocyanate method. Ethanol extract of leaves of Croton caudatum has shown effective antioxidant activity in all assay techniques. The results obtained in the present study indicate that the leaves of Croton caudatum are a potential source of natural antioxidants.
Key words: Antioxidant activity, reducing power, Croton caudatum

Introduction

Croton caudatum belonging to family Euphorciaceae growing in region of Manipur commonly called as Damdei, Lamka[1]. The plant has curative medicinal qualities for cancer, diabetes, malaria and indigestion, etc. Leaves are claimed to have anticancer properties and people from Manipur region use to take juice of leaves[3]. Presence of dotriacontamol, bamyrin and b-sitosterol in the roots and barks of the plant have been detected[3]. Croton has about 80 species all over the world.[4] Literature survey revealed that no detailed phytopharmacological work has been done on this plant and the plant leaves has yet not been screened for its evaluation of antioxidant activity of ethanolic extract along with phenolic content determination.

Fig 1: Photograph of Croton caudatum

Materials and Methods

Extraction of Plant Material

The fresh leaves of Croton caudatum were collected in the months of July-August from the local market of Amaravati, Maharashtra state, India, and authenticated by the authority of the botany department, VMV, Amaravati. A voucher specimen was submitted at Institute's herbarium department for future reference. Dried fruits were ground to coarse powder. Powder was first defatted with pet. ether and then extracted with ethanol which is further evaporated to dryness to get ethanolic extract (EE).

DPPH radical scavenging assay[5]

The free radical scavenging activity of the fractions was measured in vitro by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the fraction dissolved in ethanol at different concentrations. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a shimandzu spectrophotometer. The percentage scavenging inhibition was determined and was compared with that of ascorbic acid (AA), which was used as the standard.

Reducing power ability [5]

The reducing power was assayed as described in kuda et al.2005 with some modifications. Different

concentrations of ethanolic extracts (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 ^{o}C for 20 min. After, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. Increased absorbance values indicate a higher reducing power.

Hydrogen peroxide scavenging assay [6]

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage inhibition of different concentrations of the extracts was determined and compared with the standard, ascorbic acid.

Thiocyanate method [7]

The peroxy radical scavenging activity was determined by thiocyanate method using vit. C as standard. Increasing concentration of the fractions in 0.5 ml of distilled water was mixed with 2.5 ml of 0.02 M linoleic acid emulsion (in 0.04 M phosphate buffer pH 7.0) and 2 ml phosphate buffer (0.04M, pH 7) in a test tube and incubated in darkness at 37^{o}C. At intervals during incubation, the amount of peroxide formed was determined by reading the absorbance of the red colour developed at 500 nm by the addition of 0.1 ml of 30% ammonium thiocyanate solution and 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture. The percentages scavenging inhibitions was calculated and were compared with the standard, Ascorbic acid. A control was also prepared replacing water with plant extract

Estimation of total phenolic content [6]

The assay used for the determination of total phenolics content employs Folin and Ciocalteu's phenol reagent which response depending on the chemical structure of phenolics (i.e. the higher the number of functional –OH group the higher the total phenolics content). Total soluble phenolic compounds in the ethanolic extracts were measured and expressed as gallic acid equivalents. A sample of the ethanolic extract was added to distilled water for a final volume of 2 ml. After, it was mixed with 0.3 ml of a saturated sodium carbonate (Na_2CO_3) solution and 0.1 ml of 1 N Folin–Ciocalteu's phenol reagent. The mixture was placed for 1 h at room temperature in the dark. The absorbance was measured at 725 nm against the blank. The total phenolic content was expressed as mg of gallic acid equivalents.

Calculation of % Inhibition:

Percentage inhibition (I %) was calculated using the formula,

$$I\% = \frac{(Ac-As)}{Ac} \times 100$$

where Ac is the absorbance of the control and As is the absorbance of the sample

Results and Discussions

Phytochemical screening

Phytochemical screening of the crude ethanolic extract of the leaves of Croton caudatum revealed the presence of flavonoids, cyanogenetic glycosides, alkaloids and phenolic compounds. In addition, we could suggest that although the reducing power of a substance may be an indicator of its potential antioxidant activity, there is not necessarily a linear correlation between these two activities. The total phenolic content found to be 89.25%.

DPPH radical scavenging method

ROS produced in vivo include superoxide radical, hydrogen peroxide and hypochlorous acid. Hydrogen peroxide and superoxide can interact in the presence of certain transition metal ions to yield a highly-reactive oxidising species, the hydroxyl radical. The antioxidants react with the stable free radical DPPH (deep violet colour) and convert it to 1,1-diphenyl-2-picryl hydrazine with decoloration. The scavenging effects of extract increased with their concentrations to similar extents. The percentage inhibitions of concentration 20, 40, 60 mg/ml are about 74.23, 83.29and 94.56 % respectively (Table 1). The standard ascorbic acid presented a scavenging effect of 99.93% at the concentration of 60 mg/ml.

Table No. 1. Results of DPPH radical scavenging assay

Drug	Concentration	Absorbance
EE	20	0.189
	40	0.204
	60	0.357
Ascorbic acid	20	0.251
	40	0.289
	60	0.321

Table No. 2. Results of reducing power ability assay

Copmponds	Concentration	Absorbance	% Inhibition
EE	20	0.277	74.23
	40	0.207	83.29
	60	0.122	94.56
Ascorbic acid	20	0.245	84.12
	40	0.195	91.12
	60	0.102	99.93

Reducing power method

Table no. 2 shows the reducing power of the Croton caudatum ethanolic extracts as a function of their concentration. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. By measuring the formation of Perl's Prussian blue at 700 nm, it is possible to determine the Fe^{2+} concentration. The reducing power of the Croton caudatum ethanolic extracts increased with their concentrations. At 20, 40 and 60 mg/ml, reducing powers of both extracts were around 0.189, 0.204 and 0.357 respectively, while a solution of 40 mg/ml of ascorbic acid, the positive control used in this test, had a reducing power value of 0.321.

Hydrogen Peroxide

Extracts of leaves of Croton caudatum scavenged hydrogen peroxide in a concentration-dependent manner. The ethanol extracts of leaves of Croton caudatum showed strong H2O2 scavenging activity IC_{50} 0.276 mg/ml whereas that of the standard, ascorbic acid was 0.135 mg/ml.

Table No. 3 Results of Ferric Thiocyanate assay

Drug	Concentration	Absorbance	% Inhibition
EE	10	0.6834	65.01
	20	0.5715	72.12
	30	0.4559	86.02
Ascorbic acid	10	0.5525	74.11
	20	0.4058	89.44
	30	0.3091	98.80

Thiocyanate method

Results obtained from FTC assay (table 3) revealed that extracts of leaves of Croton caudatum carry the antioxidative potential for chain-breaking inhibition of lipid peroxidation and for free radical scavenging as extract has shown 65.01, 72.12 and 86.02% inhibition.

Conclusion

The present study suggests that leaves of Croton caudatum might be potential source of natural antioxidant.

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