

Assessment of the Radical Scavenging Activity and Total phenolic content of Crude Methanolic leaves Extract of *Aristolochia indica* L.- Aristolochiaceae

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Abstract:

The present study was aimed to screen antioxidant and quantify the phytoconstituents of *Aristolochia indica* leaves extracts evaluated the antioxidant in three methods viz. DPPH free radical scavenging activity, phosphomolybdenum method and reducing power assay. The total phenols and total flavonoids were quantitatively estimated in leaves of *A. indica*. The results thus obtained were compared with standard antioxidant compound like ascorbic acid. The results revealed that free radical scavenging activity of methanolic leaf extract of *A. indica* has radical scavenging ability on DPPH with IC₅₀ value of 38.72µg/ml. The positive control ascorbic acid showed the IC₅₀ values of 32.42µg/ml. The total phenols and flavonoids content in leaf extract indicated the antioxidant activity. The present study provides evidence that solvent extract of *A. indica* contain important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Keywords: Antioxidant, DPPH, IC₅₀ value, *Aristolochia indica*.

Introduction

Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables (Anonymous, 1988). Plant sourced antioxidants like vitamin C, vitamin E, carotenes, phenolic acids etc. have been recognized as having the potential to reduce disease risk (Anonymous, 2002). Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes. Majority of the diseases are mainly linked to oxidative stress due to free radicals. Free radicals are fundamental to any biochemical process and normal metabolism (Fang *et al.*, 2002). Antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell. Antioxidant supplements or foods containing antioxidant can interfere with oxidation process used to reduce oxidative damage in human body (Miller *et al.*, 1995; Gülçin *et al.*, 2002).

Materials and methods

Plant materials

Plants were collected from natural population

growing in the Sadhuragiri Hills forest area, Tamil Nadu, India, during October 2015. The plant sample was carried to the Botany Research Laboratory; Voucher specimen of the plant was deposited in the Botany research laboratory V.H.N.S.N. College (Autonomous) for further references.

Preparation of leaves extracts

In order to perform phytochemical analysis, 30g of dry ground plant leaves was extracting with polar methanol solvents. 30g of the dried leaves sample was taken in a conical flask and 200 ml of methanol was added. The conical flask was kept on mechanical shaker for 24 hours, after that the extract was filtered through what man No: 1 filter paper in the glass beaker and the are allowed drying in incubated in oven at 64.7°C. The dried extract was recovered and stored in Refrigerator - 4°C for further analysis. The dried plants were used for the analysis of phytochemical test.

Estimation of total phenol content

The amount of total phenol was determined with the Folin–Ciocalteu reagent using the method given by Lister and Wilson, (2001). This method was employed to evaluate the phenol content of the samples. A standard curve was prepared by using gallic acid as a standard. Different concentrations of gallic

acid were prepared in 80% of methanol, and their absorbance was recorded at 760 nm. 100 µl of sample was dissolved in 500 µl (1/10 dilution) of the Folin–Ciocalteu reagent and 1000 µl of distilled water. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1500 µl of 20% sodium carbonate (Na₂CO₃) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a UV–Vis spectrophotometer (Model. U.2800, Hitachi) and the results are expressed in mg of gallic acid equivalents (GAE) per mg of dry weight of the plant. The amount of phenol in plant extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$$X = (A. mo) / (Ao.m)$$

Where X is the phenol content, mg/mg plant extract in GAE, A is the absorption of plant extract solution, Ao is the absorption of standard gallic acid solution, m is the weight of plant extract, and mo is the weight of gallic acid in the solution.

Estimation of total flavonoid content

The flavonoid content in extracts were determined spectrophotometrically followed

by Quettier-Deleu *et al.*, (2000) using a method based on the formation of a complex flavonoid–aluminium, having the absorbtivity maximum at 430 nm. Rutin was used to make the calibration curve. 1 ml of diluted sample was separately mixed with 1 ml of 2% aluminum chloride methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a UV–Vis spectrophotometer (Model. U.2800, Hitachi) and the flavonoid content was expressed in mg per mg of rutin equivalent (RE). The amount of flavonoid in plant extracts in RE was calculated by the following formula:

$$X = (A. mo) / (Ao.m)$$

Where X is the flavonoid content, mg/mg plant extract in RE, A is the absorption of plant extract solution, Ao is the absorption of standard Rutin acid solution, m is the weight of plant extract, mg and mo is the weight of Rutin acid in the solution.

Anti-oxidant activity

Free-Radical Scavenging Ability (DPPH-assay)

The scavenging ability of methanol extract on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free- radicals was estimated according to the

method of Shimada *et al.*, (1992). This method depends on the reduction of purple DPPH to a yellow coloured diphenyl picrylhydrazine and the colour was measured at 517 nm. About 2 ml of various concentrations (10- 100µg/ml) of test sample was mixed with 0.5 mL of 0.01 mM DPPH in methanol. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and then steadily stayed for 30 min at room temperature in dark. The absorbance of the resulting solution was measured at 517 nm against the blank using a UV-Vis spectrophotometer (Model. U.2800, Hitachi). The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated according to the following equation:

$$\% \text{ DPPH radical scavenging activity} = (A_0 - A_1) / A_0 \times 100\%$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of the tested extracts. Percentage radical activity was plotted against the corresponding antioxidant substance concentration to obtain the IC_{50} value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay

system. IC_{50} values are inversely proportional to the antioxidant potential.

Total antioxidant activity by Phosphomolybdenum method

Total antioxidant capacity was expressed as ascorbic acid equivalent and was calculated using the Phosphomolybdenum method (Prieto and Pineda, 1999). Antioxidant present in the sample reduce the Mo(VI) to Mo(V) which then react with the phosphate group sodium phosphate to form a green coloured Mo(V) – Phosphate complex (Phosphomolybdenum complex) in an acetic medium. This complex is then spectrophotometrically measured at 695 nm (Model. U.2800, Hitachi). The tubes containing 0.2 ml of extract (10-200µg/ml) is mixed with 1.8 ml of distilled water, 2ml of Phosphomolybdenum reagent solution. Incubate it at 95°C for 90 minutes. The mixture is closed to room temperature and the absorbance is measured at 695 nm against reagent blank. The total antioxidant capacity was expressed as equivalents of Ascorbic Acid by using the standard Ascorbic Acid graph.

Reducing power ability

The reducing power ability of methanol extract was determined by the method given by Oyaizu (1986). Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml Potassium Ferricyanide (0.1%) and varying concentrations of extracts (10-250 µg/mL). Then the reaction mixtures were incubated at 50°C in water bath for 30 min and allowed to cool at room temperature. Then 2.5 ml of 10% TCA (Trichloroacetic acid) were added to each reaction mixture and centrifuged at 2000 rpm for 10 min. The supernatant (2.5 ml) was separated in the test tube and added with 2.5 ml of distilled water and 0.5 ml FeCl₃ (1.0%). After 10 min incubation at room temperature, the absorbance was measured at 700 nm (Model. U.2800, Hitachi). Ascorbic acid solution was used as standard. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Statistical Analysis

All analysis was carried out in triplicates. The results of scavenger activity and total phenol and total flavonoid contents were performed from the averages of all samples reading Mean \pm SD used Excel 2003.

RESULT AND DISCUSSION

Total Phosphomolybdenum method

The phosphomolybdate method is quantitative, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents. The results showed antioxidant activity in dose dependent manner at concentration 25 to 250 µg/ml. *A. indica* (138 mg/g) had a significantly higher percentage of TAC activity. Strong antioxidant activity of methanol statistically similar to ascorbic acid indicates strong antioxidants in this fraction and these could be attributable to the presence of phenolic compounds (Table,1).

Total Frap method

The frap method results showed antioxidant activity in dose dependent manner at concentration 50 to 250 µg/ml. *A. indica* (19.8 mg/g) had a significantly higher percentage of TAC activity. The frap method of total antioxidant capacity of *A. indica* has significant amount of TAC activity (Table,1).

Radical scavenging activity by DPPH method

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. DPPH method allows estimation of hydrogen radical donating ability of the extract (Sreejayan and Rao, 1996). This model represents the situation in metabolic system where an antioxidant will stabilize a free radical by reacting with the hydrogen radical. The results are expressed in IC_{50} (Inhibitory concentration to reduce the initial concentration of DPPH to 50%). Lesser the IC_{50} value for an extract is considered to be associated with higher ability to donate hydrogen radical i.e antioxidant activity. In the present study IC_{50} values were found to be $38.72\mu\text{g/ml}$ and $32.42\mu\text{g/ml}$ for the *Aristolochia indica* and Ascorbic acid respectively. These data clearly indicates that *Aristolochia indica* is a promising radical scavenger (Figure 1).

Estimation of total phenolic content

Phenolic compounds are having wide bioactivity including antioxidant properties. The antioxidant activity of phenolic compound is due to hydroxyl functional

group, however other factors eg., presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety will increase or decrease the activity. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties (Evans *et al.*, 1997). In the current study total phenolic content was found to be 31.3 mg GAE/g dw for *Aristolochia indica*. This shows that *Aristolochia indica* has high content of phenolic compounds

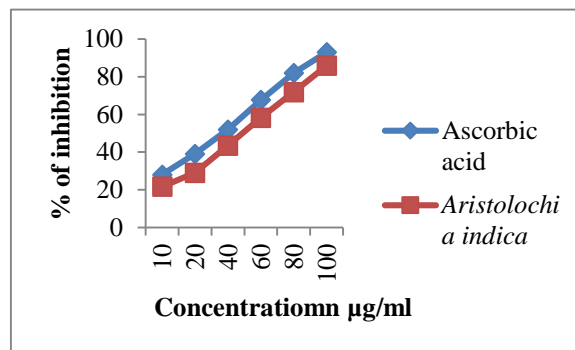


Figure: 1. DPPH free radical scavenging activity of leaf methanol extract and standard ascorbic acid.

(Where MeOH-Methanol leaves extract;
AA- Standard ascorbic acid)

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Plant	DPPH IC ₅₀ (µg/ml)	TPA (mg/g)	FRAP (mg/g)	TPC (mg GAE/g dw)	TFC(mg RAE/g dw)
<i>A. indica</i>	38.72µg	138±0.70	19.8±1.13	31.3±0.22	28±0.33

Table 1: Showing data of in vitro antioxidant activity , Total Phenolic and Flavanoid content in the methanolic leaf extract of *Aristolochia indica* (Result are expressed as mean ± S.D (n=3) from 3 independent observation, gallic acid and Ascorbic acid as standard.)

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Estimation of total flavonoid content

Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase (Li *et al.*, 1997). Flavonoids serve as health promoting compound as a results of its presence as anion radicals (Hausteen, 1983). Total flavonoid contents were found to be 28 mg RAE/g dw for *Aristolochia indica*. The compounds such as flavonoids, which hold hydroxyl groups, are responsible for the radical scavenging activity in the plants (Das and Pereira, 1997). It has been acknowledged that flavonoids

show significant antioxidant action on human health and fitness. It is known that flavonoids act through scavenging or chelating process (Kessler *et al.*, 2003; Cook and Samman, 1996). The crude methanol extract of *Aristolochia indica* leaf have indicated strong antioxidant activity which might be helpful in preventing or slowing the progress of various oxidative stress induce diseases such as diabetes, which would be beneficial to the human health. This may be related to the high amount of phenolic and flavonoid compounds present in this plant extract. We have also established the relationship of total phenolic, flavonoid contents and the free radical scavenging activity. Further studies are needed to clarify the in vivo potential of this plant in the management of human diseases resulting from oxidative stress.

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