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Research Article

PHYTOCHEMICAL, CYTOTOXIC AND ANTIOXIDANT ACTIVITIES OF LEAF EXTRACTS OF ARISTOLOCHIA INDICA (LINN.)

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ABSTRACT

Aristolochia indica is a popular medicinal plant in the Ayurvedic Pharmacopeia of India. Plant is collected from the wild for its medicinal usage, in the present study medicinal potential of cultivated medicinal plant under standard conditions was explored. Phytochemical analysis revealed presence of flavonoids and tannins like important class of compounds, where as leaf extracts of the plant showed cytotoxic as well as antioxidant potential using brine shrimp mortality and DPPH radical scavenging assays. Acetone extract of the plant showed best cytotoxic and antioxidant potential amongst all the extracts.

KEY WORDS: Aristolochia indica, DPPH, brine shrimp mortality assay, antioxidant

INTRODUCTION

Aristolochia family has wide spread distribution in tropical as well as temperate regions of the World[1]. Medicinal usage of the genus is reported from most of the Asian countries. In folk remedies roots of *A.indica* are used for the treatment of snake –bites, fevers, and minor ailments of children such as flatulence and dyspepsia[2]. The seeds are used for inflammations, biliousness, dry cough, joint pains and dyspepsia in children[3]. Majority of the medicinal plants are collected from the wild for exploiting them for their medicinal usage[4]. Medicinal importance of the plant has been from ancient times and a number of molecules have been isolated from the medicinal plants. Over exploitation of medicinal plants from wild leads to their extinction, so it is essential to bring them in the general stream of cultivation. This study was an attempt to establish the medicinal potential of the cultivated medicinal plant. So in the present study phytochemical, cytotoxic and antioxidant activities of the plant were explored of the cultivated *Aristolochia indica,* from the medicinal germplasm garden of Regional Plant Resource Centre.

MATERIALS AND METHODS

Collection and processing of plant material- Arostolochia indica leaves were collected from medicinal germplasm garden of Regional Plant Resource Centre, Bhubaneswar. *Aristolochia indica* leaves were washed properly and dried in shade at room temperature. Leaves were made into fine powder using a mechanical grinder (Lexus make).

Solvent extraction:- Solvent extraction was done using Soxhlet apparatus with solvents in the increasing order of polarity from Hexane, choloroform, acetone and methanol. Extracts were concentrated under vacuum using Buchi rotavapour. Concentrated extracts were stored in air tight screw cap vials till further use.

Phytochemical analysis-10 mg of *A. Indica* extracts were dissolved in 1 ml of different solutions (Hexane, chloroform, Acetone, Methanol), for phytochemical tests.

Phytochemical tests were conducted using the standard protocols[5]

Test for alkaloids

Dragendroffs test- 100 µl of different extract solutions were taken, 2 ml of dilute Hydrochloric acid (HCl) and 1 ml of reagent was added in each test tube. Orange brown precipitate indicates the presence of alkaloid.

Mayer'test-100 µl of different extract solutions were taken in each test tube to which 2 ml of Hydrochloric acid was added and 1ml of Mayer's reagent was added in drop wise manner. Yellow buff colour indicates the presence of alkaloids in the sample.

Wagner's test- 10 mg of different extract solution were taken, 2 ml of dilute hydrochloric acid and 1 ml of Wagner's reagent was added drop wise, reddish brown precipitate depicts the presence of alkaloids.

Test for flavonoids

Sodium hydroxide test-100 µl of different extract solutions were taken, 10% sodium hydroxide was added in drop wise manner. Yellow colour of the samples indicates the presence of flavonoids.

1% lead acetate test-100 µl of different extract solution were taken in each test tube 1% lead acetate was added in drop manner. Formation of yellow precipitate indicates the presence of flavonoids.

Test for saponins

1 ml of extract was diluted with 20 ml of water and the solution was shaken in graduated cylinder for 15 mins. Foam which is stable and remains so for more than 5 minutes indicates the presence of saponins.

Test for Tannins

Ferric chloride test-100 µl of different extract solution, 1 ml of 5% ferric chloride solution was added. The greenish black precipitate shows the presence of tannins.

Test for Steroids and Triterpenoids

Libermann –Burchard test-100 µl of different extract solution were taken, 1ml of chloroform and 1 ml of acetic anhydride were added. It was boiled, cooled and then 2-4 drops of concentrated sulphuric acid was added to it. A brown ring forms at the junction of two layer, shows the presence of steroids and Triterpenoids respectively.

Salkowski test-100 µl of different extract solution was dissolved in 2ml of chloroform and few drops of sulphuric acid was added to it. The chloroform layer if shows reddish colour and acidic layer green fluorescence/ yellow colour, then the presence of steroid and triterpinoid is established.

Antioxidant activity

TLC based antitoxidant assay

For TLC based method of Eloff et al[6] was used. Three solvents were used for TLC analysis. These were

BEA - Benzene: Ethanol : Ammonium hydroxide(90:10:1) [Non polar/Basic]

EMW - Ethyl acetate : Methanol : Water(40:5.4:4) [Polar/neutral] **CEF** - Chloroform : Ethyl acetate : Formic acid(5:4;1) [Acidic]

After developing the chromatograms, DPPH reagents was sprayed on the sheets and number of yellow bands were counted and their Rf values were calculated using the standard procedures[7].

DPPH free radical scavenging assay

A radical mixture containing 500µl of DPPH solution (1mM solution in methanol) in 200µl of methanol was taken as control. Various concentration of ascorbic acid (500, 250, 125, 62.5 µg/µl) were prepared in methanol. Then test sample were taken having concentration (5, 2.5, 1.25, 0.625µg/µl) and volume make up with methanol up to 200µl, lastly 100µl of DPPH was added to each test sample well. Then mixture was incubated in dark for 30min. at room temperature. Yellow colored chromophore was measured at 515 nm. Ascorbic acid was used as standard. The % of scavenging of DPPH free radical was calculated by following formula,

% of scavenging DPPH radical

= [(Abs control – Abs sample) Ч 100]/ Abs control

Brine shrimp mortality assay(Cytotoxic assay)

A salt solution (1.8g KCl in 100 ml distilled water) was prepared for hatching the brine shrimp larva. About 40 numbers of larva were counted and taken in each test tubes. Different doses (25, 50, 100, 200µg/ml) for each extract were tested for cytotoxic activity. The motility readings were taken after one hour difference up to 4 hours. Then the final reading was taken after 24 hrs. Percentage inhibition was calculated using the standard formula[8]. The motility readings were graded as 4+-highly mobile ,3+-motile, 2+-sluggish, 1+-non-motile, nil-dead.

RESULTS AND DISCUSSIONS

Moisture content of leaf was found to be 62 percent and methanol extract showed the maximum yield (16%) followed by hexane(4%), remaining two chloroform and acetone showed and yield of 2% each. As can be seen in Table 1, hexane and acetone extracts of *Aristolochia indica* were found to contain an important class of compound that is flavonoids. Flavonoids are well known for their anti-oxidant potential[7]. A number of reports have validated the antioxidant potential of the plant, could be due the presence of flavonoids. Methanol extract showed the presence of tannins. Tannins are basically polyphenols which are astringent in nature[10].

Antioxidant activity

Qualitative Antioxidant potential of the extracts was also explored using TLC based DPPH antioxidant assay, whereas Quantitative activity was done using DPPH radical scavenging assay. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) is a free radical and accepts an electron or hydrogen radicle to become a stable diamagnetic molecule. The intensity of the yellow color depends on the amount of radical scavenger present in the extracts. As can be seen from the Table 2, chloroform extract showed maximum number of antioxidant bands in all the three solvents. However, it showed mild antioxidant activity in

quantitative radical scavenging assay. Amongst the extracts acetone and methanol extract showed maximum antioxidant activity in quantitative radical scavenging assay (Fig 1). This study is in confirmation with previous studies [11]. Thus, it can be rightly said that cultivated plants do possess the same properties like their wild counterparts.

Table 1: Phytochemical analysis of solvent extracts of Aristolochia Indica leaves

TESTS	HEXANE	CHLOROFORM	ACETONE	METHANOL
ALKALOID TEST				
❖ **Dragendroff's test**	-ve	-ve	-ve	-ve
❖ **Mayer's test**	-ve	-ve	-ve	-ve
❖ **Wagner's test**	-ve	-ve	-ve	-ve
FLAVONOID TEST				
❖ **Sodium test**	+ve	-ve	+ve	-ve
❖ **1% lead acetate test**	+ve	-ve	+ve	-ve
SAPONINS TEST				
❖ **Foam test**	-ve	-ve	-ve	-ve
TANNINS TEST				
❖ **Ferric chloride test**	-ve	-ve	-ve	+ve
❖ **10% lead acetate test**	-ve	-ve	-ve	+ve
STEROIDS AND TRITERPENOIDS TEST				
❖ **Libermann-Burchard test**	-ve	+ve	+ve	-ve
❖ **Salkowski test**	-ve	+ve	+ve	-ve

Table 2: TLC based antioxidant assays of *Aristolochia indica* leaf extracts

EXTRACTS	SOLVENT	NO.OF BANDS	R.F Values
HEXANE	BEA	6	0.161,0.193,0.274,0.354, 0.645,0.838.
	CEF	5	0.161,0.193,0.274,0.354, 0.645,0.838.
	EMW	3	0.080, 0.193, 0.516.
CHLOROFORM	BEA	6	0.096,0.177,0.306,0.451, 0.532,0.838.
	CEF	7	.129,0.403,0.516,0.709, 0.741,0.806,0.903
	EMW	8	0.080,0.193,0.306, 0.451,0.483,0.596, 0.725,0.887.
ACETONE	BEA	2	0.112,0.274
	CEF	4	0.370,0.677,0.790,0.887.
	EMW	2	0.741, 0.854.
METHANOL	BEA	streak	
	CEF	4	0.387,0.693,0.806,0.903.
	EMW	2	0.725, 0.870

Figure 1: Quantitative DPPH Assay

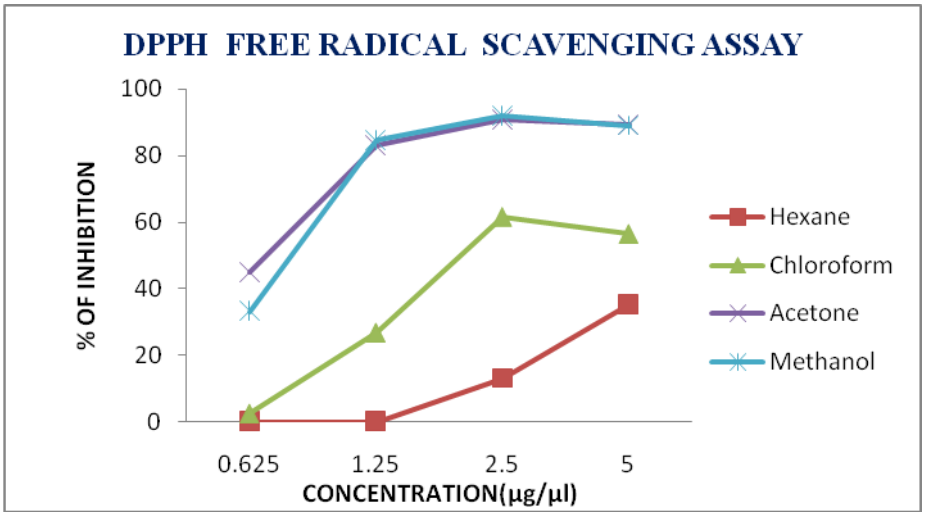
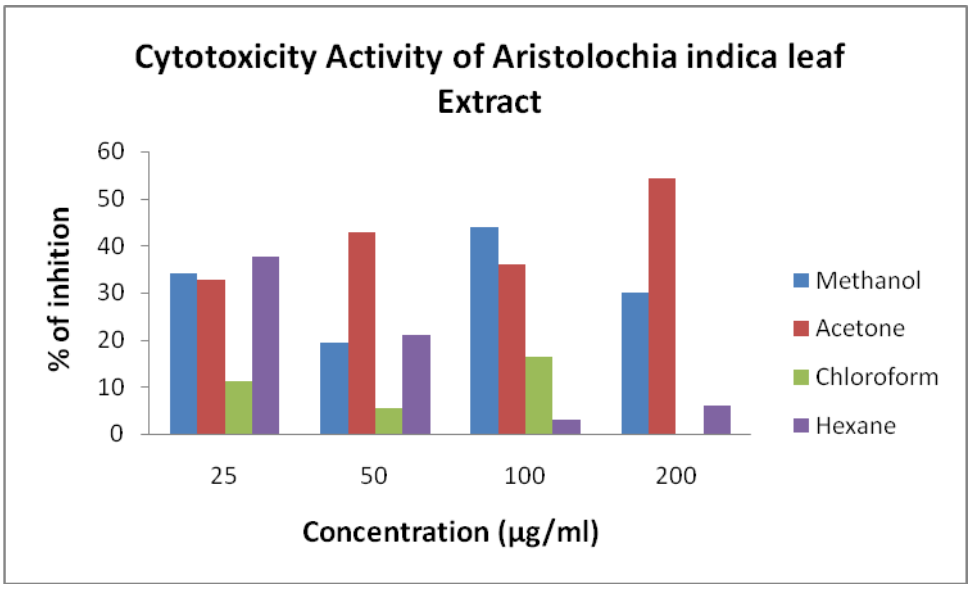


Figure 2: Cytotoxic assay of A.indica leaf extracts



Cytotoxicity Testing

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties [12] In the present study, cytotoxic activity was observed by motility of brine shrimp for 4hrs and compared with that of controlled samples. It was observed that up to 4hrs there was no effect on the larvae. Their motility was comparable with the control samples but after 24hrs dose dependent activity was noted. Amongst the four extracts acetone extract was most active at a higher dose of 200microgram/ml(Fig 2). Amongst all the extracts Acetone extract showed antioxidant as well as cytotoxic activity and hence needs to be further explored.

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REFERENCES

[1] Kirtikar KR, Basu BD. Indian medicinal plants Vol III.Periodical Experts, New Delhi, 1975; 2120-2125.

[2] Sati H, Sati B, Saklani S, Bhatt C, Mishra AP. Phytochemical and Pharmacolology potential of Aristolochia indica: A review. *Res Jou Pharmacol Biol Chem Sci.* 2011, 2: 647-653.

[3] Jayasutha J, Nithila MJS. Evaluation of wound healing activity of ethanol extract of A. bracteata Cassia tora on wistar albino rat. *Int J PharmaTech Res.* 2011, 3: 1547-1550.

[4] The Wealth of India: Dictionary of India Raw materials and industrial products-Raw material series, Publications and information Directorate, Council of Scientific & Industrial search, New Delhi Vol I: 88.

[5] Harborn JB: Phytochemical Methods: A guide to Modern Techniques of plants Analysis, Chapman & Hall. London, Ltd; 1973: 49-188.

[6] Eloff, LN, Famakin, JO and Katerere, DP :Isolation of antibacterial stilbene from combretum woodii (Combretaceae) leaves. *African Journal of Biotechnology*,2005, 4:1166-1171

[7] Bhatnagar S, Sahoo S, Mohapatra AK and Behera DR: Phytochemical analysis, Antioxidant and Cytotoxic activity of medicinal plant *Combretum roxburghii* (Family: Combretaceae). *International journal of drug development and research*, 2012, 4: 193-202.

[8] Mohapatra, R., Bhatnagar, S. and Das, P. Filaricidal activity of a wild herb, Typhonium trilobatum. Advances in Plant Sciences, 2010; 23, 51-2.

[9] Kumar, S, Pandey AK, Chemistry of biological activities of flavonoids: An over view. Scientific World Journal, 2013; Artid.162750. 1-16.

[10] Saxena V, Mishra G, Saxena A, Vishwakarma KK. A comparative study on quantitative estimation of tannins from Terminalia chebula, T. bellerica, T. arjuna and Saraca indica using spectrophotometer. 2013, 6: 148-149.

[11] Hossen JMM, Hossain MS, Islam J, Pinto MN, Jannat NE, Ahmed F. Comparative preliminary phytochemical and biological investigation on Andrographis paniculata and Aristolochia indica. *Der Pharma Chemica.* 2014, 6: 332-338.

[12] Meyer BN, Ferrigni NR, Putnam JE ; Brine shrimp: a convenient general bioassay for active plant constituents; Planta Med ; 1982, 45: 1-34