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Comparative preliminary phytochemical and biological investigations on *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn)

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ABSTRACT

Foremost objective of the present research was Phytochemical and biological activity investigations of methanolic extract of Aristolochia indica (Linn.) (Aerial part) and leaves of Andrographis paniculata (Nees). The identifications of phytochemical carried out by following standard chemical procedure. Anti-oxidant activity screening was performed by free radical scavenging method, anti-bacterial activity by disc diffusion method and cytotoxic activity by brine shrimp lethal bioassay. The results showed the plant extract contains many phytochemical compounds. Methanolic extract of Andrographis paniculata (Nees) showed strong antioxidant and cytotoxic activity. On the other hand, Aristolochia indica Linn showed moderate cytotoxic activity and mild antioxidant. The present study Andrographis paniculata (Nees) showed strong anti oxidant and cytotoxic activity suggests future higher research study. The plant available in Bangladesh and could be prominent source of medically important natural compound.

Keywords: *Andrographis paniculata* Nees, *Aristolochia indica Linn*, Antioxidant, Anti-Bacterial and Cytotoxic.

INTRODUCTION

Andrographis paniculata belongs to family Acanthacea. Synonyms**:** Justicia paniculata Burm.f). Bengali/Vernacular Name is Kalomegh, Kalamegh and tribal name is Kalpanas (Garo). English name **is** Creat. The plant is febrifuge, hypoglycemic, stomachic, tonic, alterative, anthelmintic and cholagogue. Used in general debility, dysentery, certain forms of dyspepsia and in liver complaints, mainly of children. It is a domestic medicine for flatulence and diarrhoea of children. The plant is also used in spleen complaints, colic and strangulation of intestine, constipation, diarrhoea, cholera, phthisis and consumption. Juice of the leaves together with spices, such as cardamoms, cloves, cinnamons, etc., is dried in sun, and made into little globules, which are prescribed for infants to relieve griping, irregular stools and loss of appetite. The plant is used for malaria in Rema-Kalenga. The Garo of Madhupur prescribes leaf juice against fever, headache and vertigo. Leaf extract is hypotensive. Powdered stem showed anti-fertility activity in male mice [1]. Andrographolide contained in the plant possesses strong anti-inflammatory and also cytotoxic activity [2]. Both aqueous and ethanolic extract of the plant possesses significant blood sugar lowering effect in both glucose-loaded and alloxen induced diabetic rat [3]. The plant shows antimalarial [4], anti-inflammatory, antioxidant [5], antihepatitic [6], antihyperglycemic [7], anthelmintic [8], antibacterial [9], antipyretic [10] and anticancer activity [11].

On the other hand, *Aristolochia indica* (family: Aristolochiaceae). Bengali/Vernacular name is Iswarmul. English Name is Indian Birthwort. Two new sesquiterpene hydrocarbons ishwarane and aristolochene from roots and structure of a tetracyclic sesquiterpene ishwarone was determined. Ishwarol isolated from roots and its structure was established [12]. A new sesquiterpene hydrocarbon (I) isolated and characterized as 5β-H, 7β, 10α-selina-4(14), II-diene [13]. A phenanthrene derivative Aristololactam N-β-D-glucoside and two steroids 3β-hydroxy-stigmast-5-en-7-one and 6β-hydroxy-stigmast-4-en-3-one was isolated from A.indica [14]. The roots contain aristolindiquinone, aristolide, 2-hydroxy-1-methoxy-4Hdibenzo quinolone-4,5-(6H)-dione, cephradione, aristolactam IIa, stigmastenones II and III, methylaristolate, β-sitosterol-β-D-glucoside aristolactam glycoside I, ishwarol, ishwarone, methylaristolate and aristolochene [15-16]. A new naphthoquinone Aristolindiquinone [17], Aristolochic acids and Aristolactams [18] was reported from A. indica. The aqueous extract of the roots of *A. indica* is used as a decoction for the ailment of snake bite treatment [19].

Andrographis paniculata (Nees) and *Aristolochia indica* (Linn) are most common traditional medicinal plants. Our present study aims to reveal antioxidant (a search for natural antioxidant), cytotoxic (to determine the safety profile for use) and antibacterial property of locally growing *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn).

MATERIALS AND METHODS

Plant collection:
Andrographis paniculata (Nees) and *Aristolochia indica* (Linn) were collected from at Natore in Rajshahi. The leaves were collected in January, 2014 during day time. The collected leaves were fresh and the mother tree was adult. The leaves were collected without any adulteration. The plants were authenticated by Mr. Mohammad Omar Faruk, Lecturer, Department of Botany, Chittagong University.

Extraction of plant material:
700gm powder of leaves *Andrographis paniculata* (Nees) and aerial part of *Aristolochia indica* (Linn) ware taken in a clean flat bottomed glass container and soaked in 2000 mL methanol. The container with contents was sealed and kept for a period of 15 days upon accompanying occasional shaking and stirring. The whole mixture was coarse filtered by clean, white cotton and then followed a filtration through Whatmann filter paper. After filtrations they were drayed by the rotary evaporator. Finally a blackish crude extract was obtained.

Phytochemical screening:
The crude extract of both plants were used for qualitative phytochemical analysis, was aim to determine the presence of alkaloids (Mayer's and Dragendroff's test), glycoside (Killer-killani's test) steroids (Sculptures acid test), tannins (Ferric chloride and Potassium dichromate test), Flavonoids (Modified Ammonia test) and saponin (Frothing Test) [20].

DPPH free radicals Scavenging Activity:
The free radicals scavenging activity of both plants extract were measured by decreased the absorbance of methanolic solution of DPPH (2,2-Diphenyl-1- picrylhydrazyl). The method modified by Gupta *et al*., [21, 22]. Stock solutions (1 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 µg/mL. Diluted solutions (2 mL) were added to 2 mL of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against concentration and from the graph IC_{50} was calculated. The experiment was performed in duplicate and average absorption was noted for each concentration. Ascorbic acid was as positive control.

Brine Shrimp lethality bioassay:
The brine shrimp lethality bioassay used to determine the cytotoxic activity of the extract. The test was performed by hatching 5mg of eggs of 1000ml of artificial sea water after incubation at 37°C for 48h with continuous oxygen's supply. The nauplii were allowed another 48h in seawater to ensure survival and maturity before use. Five doses of plant extract (10, 20, 40, 80 and 160 µg/mL) in 5% DMSO was prepared. Each extract preparation was dispensed into clean test tube 10mL volumes and tested in duplicates. The concentrations of DMSO in the vials were kept below 10µL/mL. For control same procedure was flowed. After marking the test tubes properly, 10 living nauplii

were added to each of the 20 vials with the help of the Pasteur pipette. The test tube containing the sample and control were then incubated at 37°C for 24h in a water bath, after which tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration [23- 26].

Anti-Bacterial Activity:
The anti-bacterial activity performed by following the disc diffusion method by using 100 µg/ml of extract solution. The microorganisms spread on nutrient ager medium for sub culture then preparation of seeded test plates. Dried and sterilized filter paper discs (4-5mmin diameter) and other equipment. Placement of Sample disc, Standard disc & Blank disc on seeded plates. Sample disc used 500 µg/disc of extract solution, Standard disc used of ciprofloxacin (30µg/disc) and blank discs were used as negative and positive control. After incubation at 37°C for 24 hours, the antimicrobial activity of test agents were determined by measuring the diameter of zone of inhibition expressed in mm [27].

RESULTS

Phyto-chemicals screening:
Results of different group tests of the selected plants *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn) summarized below.

Constituents	*Andrographis paniculata*	*Aristolochia indica*
Alkaloids	+	+
Glycosides	-	+
Saponins	-	+
Tannins	+	+
Steroids	-	-
Flavonoids	++	+

+ = present *- = Absence*

DPPH free radical scavenging activity:
Methanolic extracts of the *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn) showed strong antioxidant activity where the IC_{50} was 31.18 µg/mL, against DPPH free radical.

Table 1: Evaluation of antioxidant activity of the collected plants

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
MeOH extract of *Andrographis paniculata*	1	1±0.01	11.47
	5	21±0.01	
	10	60±0.001	
	50	95±0.025	
	100	95±0.018	
	500	96±0.01	
MeOH extract of *Aristolochia indica*	1	0±0.011	223.63
	5	3±0.013	
	10	0±0.011	
	50	11±0.035	
	100	29±0.028	
	500	85±0.041	
Ascorbic acid	1	21±0.016	7.8
	5	27±0.017	
	10	55±0.019	
	50	97±0.045	
	100	97±0.038	
	500	97±0.051	

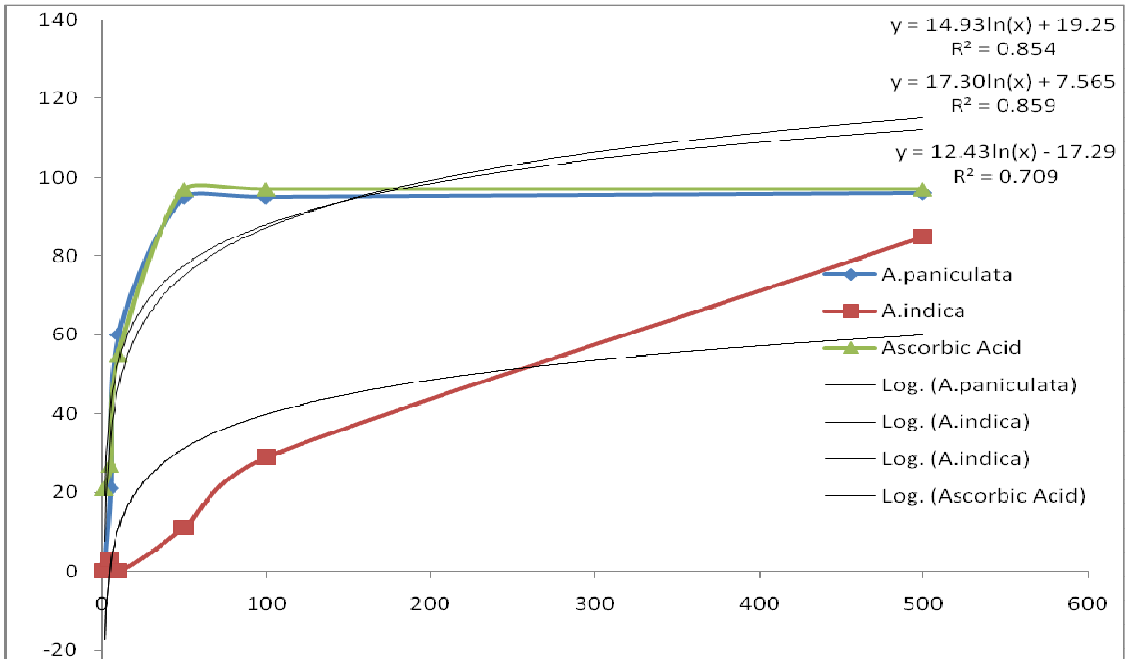

Fig. 1: Calibration curve of *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn.)

***In vitro* Antibacterial activity:**

Methanolic extracts of the *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn.) showed mild Antibacterial activity.

Table 2: *In vitro* Antibacterial activity of *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn.)

Bacterial strains		Diameter of zone of inhibition (mm)		Ciprofloxacin (30 µg/disc) Mean ± SD
		Andrographis paniculata (500 µg/disc) Mean ± SD	*Aristolochia indica* (500 µg/disc) Mean ± SD	
Gram positive	*Bacillus subtilis*	7.02 ± 0.11	**R**	30.01 ± 0.31
	Bacillus cereus	6.11 ± 0.15	6±0.15	32.21 ± 0.33
Gram negative	*Pseudomonas aureus*	**R**	**R**	35.06 ± 0.36
	E. coli	6.14 ± 0.18	**R**	35.04 ± 0.34

R= Resistant / No growth.

Brine Shrimp lethality bioassay:

Methanolic extracts of *A.paniculata and A.indica* Showed strong Cytotoxic Activity in Brine Shrimp bioassay lethality test.

Table 3: Brine Shrimp lethality bioassay of *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn.)

Con.	Log Conc.	% Mortality *Andrographis paniculata*	LC_{50} (µg/ml) Mean±SD *Andrographis paniculata*	% Mortality *Aristolochia indica*	LC_{50} (µg/ml) Mean ± SD *Aristolochia indica*
10	1	40	18.450 ± 0.86	30	36.31 ±0.43
20	1.301	50		40	
40	1.602	60		50	
80	1.903	80		70	
160	2.204	100		70	
320	2.505	100		80	

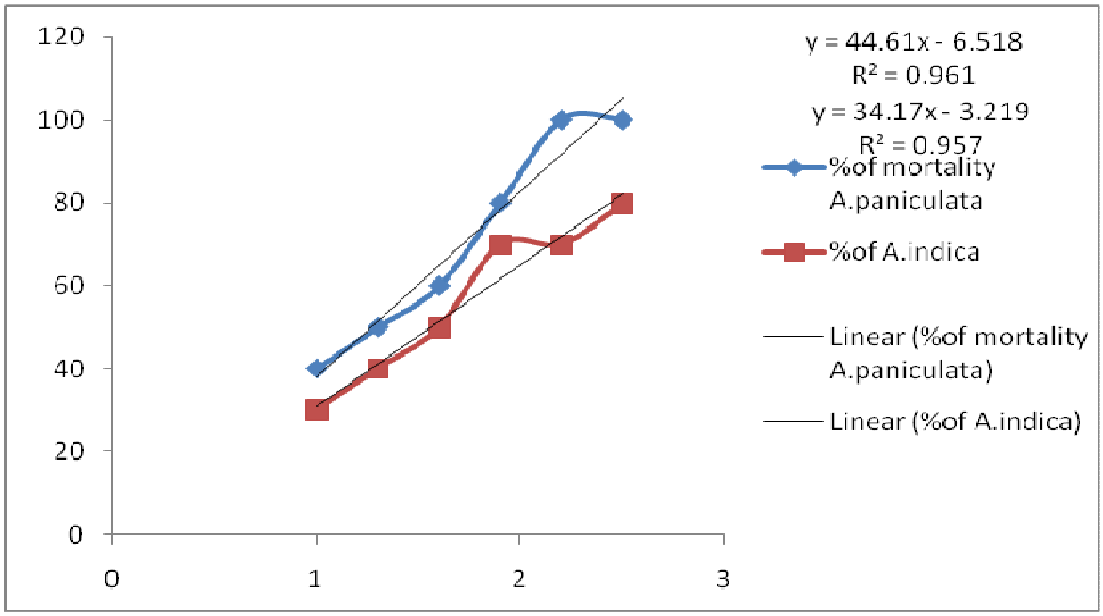

Figure 2: Calibration curb of Brine Shrimp lethality bioassay

DISCUSSION

Andrographis paniculata (Nees) and *Aristolochia indica* (Linn) both are most common and popular traditional medicinal plants of Bangladesh. Both are phytochemically rich and have diversified traditional use. Preliminary phytochemical screening confirms that *Andrographis paniculata* (Nees) contain alkaloids, tannins and flavonoids and *Aristolochia indica* (Linn) contain alkaloids, glycosides, saponins, tannins and flavonoids. Both plants differ from each other due to glycosides and saponin content. Glycosides from *Aristolochia indica* (Linn) may be flavonoidal glycosides.

Based on the DPPH free radical scavenging activity the extract of *A.paniculata* showed a strong activity due to the presence of large amount of Flavonoids and Tannins. On the other hand, *A.indica* has mild activity. Phytochemical screening shows that flavonoids content of Andrographis paniculata is more than Aristolochia indica. Polyphenolic compounds, like flavonoids, tannins and phenolic acids have the antioxidant activity. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [28, 29]. An antioxidant is any substance that, when present at low concentrations significantly prevents oxidation of cell content like protein, lipid, carbohydrates and DNA.

Extracts are regarded as non-toxic if its LC_{50} is greater 100µg/mL in brine shrimp lethality assay [30]. The mortality percentage and LC_{50} (lethal concentration for 50% of the population) were determined using statistical analysis and the graph of logarithm of concentration against percentage lethality [31]. Methanol extract of *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn) has moderate cytotoxicity in comparison to standard sample (Figure 2). According to the literature, compounds that present brine shrimp (Artemia salina) toxicity, in general also have cytotoxic properties against cells of solid tumors found in humans [32]. The presence of flavonoids, glycosides, alkaloids and saponin in plants probably responsible for this activity because the biological activities of plants may be due to the presence of these diverse group of chemical compounds [33].

Methanol extract of *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn) showed very negligible effect against the Gram positive and Gram-negative organisms.

CONCLUSION

Andrographis paniculata (Nees) and *Aristolochia indica* (Linn) both plants are moderate cytotoxic in nature and may be a good source of cancer chemotherapeutic agent. This research suggest us further research and compound isolation and identification is necessary.

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