IN-VITRO STUDY ON CYTOTOXIC ACTIVITY OF *ARISTOLOCHIA INDICA* AND *ARISTOLOCHIA BRACTEATA* USING BRINE SHRIMPS

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Summary

Among recent advanced research in cancer chemotherapy, phytochemicals play significant role as chemotherapeutic drugs. Investigation for new anti-cancer drugs has taken many different approaches. The brine shrimp hatchability test and lethality bioassay are efficient, rapid and inexpensive tests that require relatively small amount of samples and suggests that the procedure can also be extended for evaluating cell line toxicity and anti-tumor activity. In the present study two medicinally important South Indian plants *Aristolochia bracteata/bracteolata* Lam and *Aristolochia indica* Linn. were evaluated for cytotoxicity using brine shrimp (*Artemia salina*) lethality and hatchability tests. All the extracts exhibited cytotoxic activity. Alcoholic extract of stems of *Aristolochia indica* was found to be more potent and elicited 100% hatching inhibition at 10mg/ml concentration and LC_{50} of 2.4 mg /ml followed by alcoholic stem extract of *Aristolochia bracteata* which showed 100% hatching inhibition at 20 mg /ml and LC_{50} of 5.2 mg /ml The cytotoxic activity may be attributed to the presence of alkaloids and steroids which were found in the extracts. The result reveals that the plants contain potential cytotoxic compounds and study of these compound/s in detail may be helpful to develop novel antitumor lead compounds.

Key Words: *Aristolochia bracteata*, *Aristolochia indica*, Cytotoxic, Antitumor

Introduction

Current research in cancer chemotherapy reveals the role and importance of phytochemicals in the treatment of cancer and tumors. A search and evaluation for new anti-cancer/anti tumor drugs has taken many different approaches. The brine shrimp lethality bioassay by Meyer et al. is one of the efficient, rapid and inexpensive methods which require relatively small amount test samples[1]. A positive correlation exists between brine shrimp lethality and human carcinoma[2] The method can also be extrapolated for cell line toxicity and anti-tumor activity[3] . *Aristolochia indica* Linn.(Indian birthwort) and *Aristolochia bracteata* Ram. are commonly available South Indian medicinal plants belonging to the family Aristolochiaceae. The plant has a number of historical medicinal uses. Both plants contain aristolochic acid a rodent carcinogen and in Ayurveda the

leaves, seeds and the roots of *Aristolochia bracteata* are used for treatment of skin diseases, ulcers eczema and as vermifuge [4]. Chloroform fraction of roots of *Aristolochia indica* was reported to be interceptive in female mice [5]. Alcoholic extract of the roots are reported to be anti-tumor (Against adenocarcinoma 755 in mice)[6] Oil from roots of *Aristolochia indica* was found to be moderately antibacterial[7] *Aristolochia bracteata* is reported to be wound healing[8] and antiallergic[9]. Based on these reports this study has been undertaken to evaluate and compare the cytotoxic potential of both the plants

Method

Collection and authentication of plant material: Plants *Aristolochia indica* and *Aristolochia bracteata* were collected from the local garden, Shimoga District, Karnataka in the month of July 2009 and were authenticated by a botanist, Gouthami Kala Shala, Ibrahimpatnam Voucher specimens Ar1-09 and Ar2-09 respectively are preserved in our department for further reference.

Preparation of the plant extracts
Plant materials were washed thoroughly and dried in oven at 60^0 . Roots, stems and leaves were separated and powdered. 10g of each powder was refluxed with 50ml of 70% alcohol for six hours to prepare alcoholic extract. Extracts were filtered through Whatman No.1 filter paper and concentrated to dryness at low temperature. Aliquots were prepared from the dried extracts using DMSO (Dimethyl Sulfoxide to give desired concentrations of 2mg/ml, 5mg/ml. 10mg/ml and 20mg/ml

Determination of cytotoxicity:
Brine shrimp lethality and hatchability assay by Meyer et.al[10] and Joes Louis Carbello et.al[11]. with little modification was adopted to study the cell toxicity of the extracts. . Assay was carried out in triplicates.

Brine Shrimp Hatchability Assay: Brine shrimp hatchability assay was carried out using the hatchability efficiency of *Artemia salina* against different concentration of *Aristolochia bracteata* and *Aristolochia indica* plant extracts. Procedure was standardized as per Joes Louis Carbello et.al[11]. About 0.5 g of dried cysts were separated from their shells using the commercial brine shrimp hatchers solution. After that, the cysts were hatched in seawater (500ml) at 28°C, under conditions of continuous illumination and strong aeration. After 2 h aliquots measuring 500 µl were placed in each well where the extracts ranging from 2mg/ml-20mg/ml had previously been placed and they were incubated at the same conditions of temperature and illumination under gentle shaking. After 48 h of exposure the free nauplii were counted under a stereomicroscope. The percentages of hatchability was calculated by comparing the number of free nauplii in the treatment with the number of free nauplii in the control. The percentage of hatch inhibition (%HI) was calculated as: % HI = % hatchability in the control - % hatchability in each treatment.).Results of % inhibition shown by the extracts are tabulated in Table1

Brine Shrimp Lethality Test: 1g of dried cysts in artificial sea water (in glass jar) was incubated at room temperature with aeration, under a continuous light regime for two days. After 48hrs free swimming phototropic nauplii were collected from the bottom surface of the jar with a pipette and transferred to small vials. Each test consisted of exposing groups of 20 Artemia to various concentrations of the extracts and standard compounds. The toxicity was determined after12hrs of exposure. The numbers of survivors were counted with the help of hand lens and percentage of deaths was calculated..

The larvae was not fed with any food. To ensure the death of the larvae is due to the active components in the extracts and not because of lack of food, the dead larvae in each test treatment was compared with the control. It was observed that, hatched brine shrimp nauplii can survive for up to 48 h without food because they still feed on their yolk-sac. The percentage of mortality (% M) was calculated as: % M = percentage of survival in the control - percentage of survival in the treatment[11].

LC_{50} values were calculated graphically (manually) by plotting concentration of extracts *vs* percentage mortality in the extracts (interpolation method). Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation. Results of LC_{50} are tabulated in Table 1

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Results and discussion

The present study reveals the importance of the plants *A. indica and A. bracteata* as cytotoxic. From a pharmacological point of view, a good relationship has been found with the brine shrimp lethality test to detect antitumoral compounds in terrestrial plant extracts[12] .Cytotoxicity was determined using Brine Shrimps lethality assay and hatchability test. All the extracts exhibited cytotoxicity in which alcoholic extract of stems of *Aristolochia indica* was found to be more potent and elicited 100% hatching inhibition at 10mg/ml with LC_{50} of 2.4 mg /ml followed by alcoholic extract of *Aristolochia bracteata* stem extract which showed 100% hatching inhibition at 20 mg /ml and LC_{50} of 5.2 mg /ml The cytotoxic activity may be attributed to the alkaloids and steroids which were found the extracts. From the data obtained it can be predicted that the plants contain potential cytotoxic compounds which possess tumour reduction potential and study of these compound/s in detail will be helpful to develop novel antitumour lead compounds.

Table 1. Percentage hatching inhibition and LC_{50} values exhibited by *Atistolochia bracteata* and *Aristolochia indica* plant extracts

Test compounds	Concentration mg/ml	Brine shrimp hatchability assay * % of hatching inhibition	Brine Shrimp Lethality assay LC_{50} mg/ml
Control	---	0.0	---
A.indica			
Root extract	2.0 5.0 10.0 20.0	42.2 51.9 82.8 100.0	2.9
Leaf extract	2.0 5.0 10.0 20.0	46.2 62.8 88.6 100.0	2.5
Stem extract	2.0 5.0 10.0 20.0	48.2 88.9 100.0	2.4
A. bracteata			
Root extract	2.0 5.0 10.0 20.0	32.2 48.9 56.8 75.0	5.1
Leaf extract	2.0 5.0 10.0 20.0	44.2 55.8 72.4 94.6	2.92
Stem extract	2.0 5.0 10.0 20.0	40.4 48.8 80.6 100.0	5.2

* Results are average of three observations

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