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BIOLOGICAL EVALUATION OF CRUDE ALKALOID EXTRACTS OF *NYCTANTHES ARBORTRISTIS* AND *ARISTOLOCHIA INDICA*

Usha Rani Pradhan, Sumit Sarangi and Sunita Bhatnagar*

Senior Scientist, RPRC, Bhubaneswar.

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***Corresponding Author**
Sunita Bhatnagar
Senior Scientist, RPRC,
Bhubaneswar.

ABSTRACT

Nyctanthus arbortristis and *Aristolochia indica* are popular plants in the Ayurvedic system of medicines. In the present study two different protocols of alkaloid isolation from the leaves of the plant were taken up and their biological activity was evaluated using brine shrimp assay and Thin liquid chromatographic analysis. Crude alkaloids isolated from the second protocol showed 100% activity against brine shrimp larvae in case of both the plants. All the extracts showed chromatographic separation in different solvents, best separation was obtained in benzene solvent.

KEYWORDS: *Nyctanthus arbortristis*, Alkaloids, brine shrimp assay, TLC.

INTRODUCTION

Nyctanthes arbortristis is commonly known as Harsingar is widely used in Ayurvedic system of medicine for the treatment of sciatica, arthritis, fevers, various painful conditions and as laxative.[1] Nyctanthes arbor-tristis L. (Oleaceae), a plant widely used in the traditional medicinal systems of India, has recently been reported to possess hepatoprotective, antileishmanial, antiviral and antifungal activities.[2] The leaves of *Nyctanthes arbor tristis*, besides being used in the treatment of sciatica and arthritis, are advocated for various kinds of fevers and painful conditions by the Ayurvedic physicians.[3] The seeds of the plant are also useful in piles and skin diseases.[4] Leaves have been reported to possess an alkaloid useful in malaria fever.[5] *Aristolochia indica* is a creeper belonging to family Aristolochiaceae is used in traditional medicines as a gastric stimulant, in the treatment of cancer, lung inflammation, dysentery and snake bites.[6] It is a native of India and distributed thorough out the country. Roots contain the necessary medicinally active components which are utilized in Ayurvedic, Chinese and other traditional medicines.[7] All the plants in which roots are the medicinally

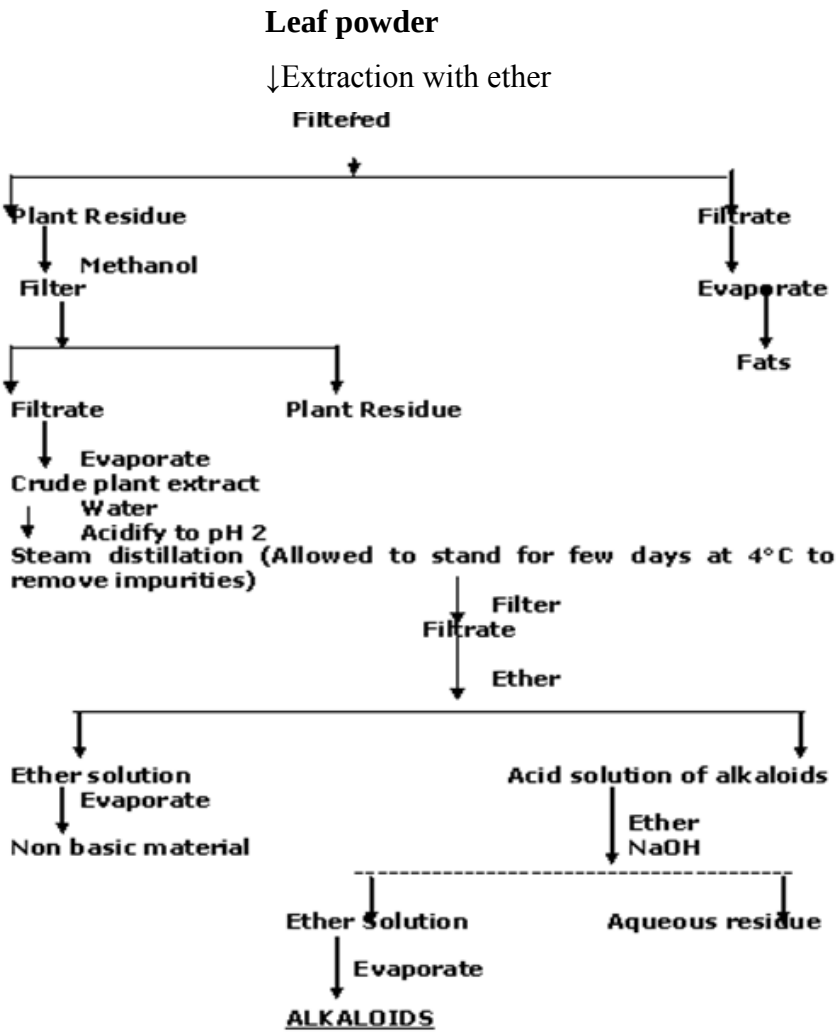
important parts are very badly exploited, for collecting roots complete plant is rooted out with the result they become endangered. In the present study the medicinal importance of leaves of the plant was explored. In the present study two protocols for alkaloid isolation were followed and their biological activity was studied on brine shrimp larvae which is a good indicator of cytotoxic activity.[8]

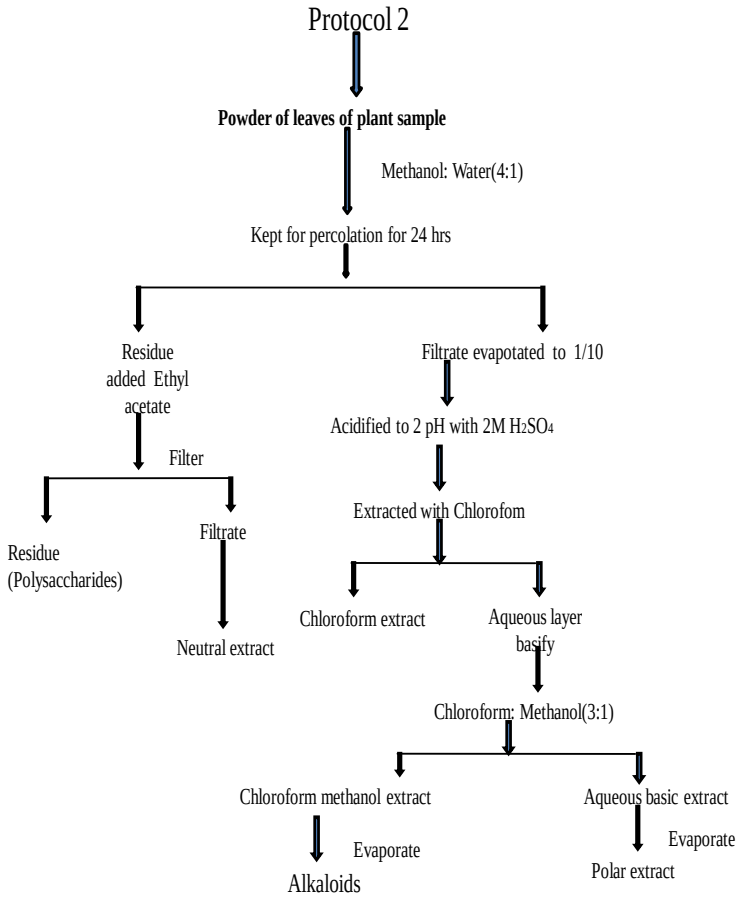
MATERIALS AND METHODS

Plant collection: Leaves of both the plants Nyctanthes *arbortristis* and *Aristolochia indica* were collected from the medicinal germplasm garden of Regional Plant Resource Centre, Nayapalli, Bhubaneswar. Leaves were dried in shade. After drying leaves were powdered by lexus grinder. Leaf powder was utilized for the preparation of crude alkaloid extracts using two standard protocols.[9,10]

Brief outline of both the protocols are shown below:

Protocol 1





Bioevaluation of extracts against artemisia brine shrimp larvae

Brine shrimp motility assay was conducted as per the standard protocol.[11] Brine shrimp eggs were procured from East Coast Marine Company, India. Eggs were kept for hatching in 6% normal saline for 18hrs. Plant extracts obtained by following the above protocols were subjected to motility assay. Readings were taken every hour upto four hours and later at 24 hrs. Motility was graded as below:

4+ = Highly motile

3+ = Motile

2+ = sluggish

1+ = slow

Nil = No activity at all.

Thin layered chromatography

In order to study the separation of compounds different solvent like Hexane, benzene, chloroform, ethyl acetate, Butanol and acetonitrile were used as solvents. TLC plates were prepared on 15 cm slides using Silica gel G of SD fine chemicals by standard protocols. Activated and used for chromatography for other solvents. Rf factor was calculated using the following formula:

Retention Factor (Rf) = Distance travelled by the sample X 100

Distance travelled by the solvent

RESULTS AND DISCUSSIONS

Both the protocols revealed a total of 6 extracts 2 in protocol 1 and 4 in protocol 2. All the extracts were subjected to Thin layered chromatographic analysis and brine shrimp assay. TLC gives an insight into the number of prominent molecules[12] whereas brine shrimp motility assay gives the toxicity/ activity potential of the extracts.[13] As can be seen from the Table 1, *Nyctanthes arbortristis* exhibited a number of bands in majority of the solvents whereas in *Aristolochia indica* only crude alkaloid extract showed two bands one in benzene solvent and other in hexane solvent suggesting very limited number of molecules in the extracts.

However, all the extracts obtained in Protocol 2 exhibited very good separation in thin layered chromatography analysis as can be seen from Tables 2 and 3. In number of cases streak was obtained which suggests large number of molecules situated very closely. Neutral and chloroform extracts showed more than three bands in benzene solvent. Thus, clearly study has indicated that Protocol 2 has more potential of isolation of molecules as number of bands were more in the extracts.

Table 1: TLC analysis of extracts of Protocol 1.

Solvent	*Nyctanthes arbortristis* Rf factors		*Aristolochia indica* Rf factors	
	Crude alkaloid extract	Aqueous extract	Crude alkaloid extract	Aqueous extract
Acetonitrile	Nil	54.4, 66.2, 82.4.	Nil	Nil
Benzene	69.23, 51.9	18.3	50	Nil
Butanol	Nil	80	Nil	Nil
Chloroform	77.7	21.8, 29, 41.2	Nil	Nil
Ethyl acetate	63.5, 80.76	25, 41.7, 61.1	Nil	Nil
Hexane	Nil	Nil	76.78	Nil

Table 2: TLC analysis of extracts of *Nyctanthes arbortristis* (Protocol 2).				
Solvent	**Neutral extract**	**Chloroform extract**	**Aqueous extract**	**Crude Alkaloid extract**
Acetonitrile	Streak	Streak	36.07	Streak
Benzene	21.88, 29.68, 40.6	13.3, 26.7, 36.7, 53.3, 70	20	Nil
Butanol	45.16, 66.13	93.54	81.81	Streak
Chloroform	Streak	56.14, 73.68	Streak	66.67
Ethyl acetate	Streak	Streak	91.94	62.9, 74.19
Hexane	Nil	Streak	Nil	Nil

Table 3: TLC analysis of extracts of *Aristolchia indica* (Protocol 2).				
Solvent	**Neutral extract**	**Chloroform extract**	**Aqueous extract**	**Crude Alkaloid extract**
Acetonitrile	10.6, 50, 71.2	Streak	61.1	Streak
Benzene	28.7,45.4, 57.5	Streak	Nil	31.7
Butanol	Streak	51	69.1	75.3
Chloroform	26.8, 95.5	10.2, 14.6, 20.5, 44.1, 82.3	Nil	31.03, 34.8
Ethyl acetate	51.5	32.3, 43.07, 60	40.4	37.7
Hexane	50	Nil	Nil	Nil

Brine shrimp motility assay

This assay has also suggested that protocol 2 has more potential when compared with the first one. In case of protocol one(Table 4) aqueous extract of *Nyctanthes arbortristis* showed mild activity(3+), other extract failed to exhibit any cytotoxic potential. Further as can be seen from Table 5, Crude alkaloid extracts of both the plants along with the aqueous extract showed 100% reduction in the motility of brine shrimp larvae which suggests that same extracts need to be explored for anticancer and anti inflammatory activity as brine shrimp assay has a good co relationship with both the above activities.[14]

Table 4: Bioevaluation of crude alkaloids and aqueous extract of *Nyctanthes arbortristis* and *Aristolochia indica* (Protocol 1)							
Plant	**Group**	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**24hrs**
Nyctanthes arbortristis	Control	4+	4+	4+	4+	4+	4+
	Crude alkaloid extract	4+	4+	4+	4+	4+	4+
	Aqueous extract	4+	4+	4+	**3+**	**3+**	**3+**
Aristolochia indica	Crude alkaloid extract	4+	4+	4+	4+	4+	4+
	Aqueous extract	4+	4+	4+	4+	4+	4+

Table 5: Bioevaluation of 4 extracts obtained after protocol 2.							
Plant	**Group**	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**24hrs**
	Control	4+	4+	4+	4+	4+	4+
Nyctanthes arbortris tis	Neutral extract	4+	4+	4+	4+	4+	4+
	Chloroform extract	4+	4+	4+	3+	4+	4+

	Crude alkaloid extract	4+	4+	Nil	Nil	Nil	Nil
	Aqueous extract	4+	4+	Nil	Nil	Nil	Nil
Aristolochia indica	Neutral extract	4+	4+	4+	4+	4+	4+
	Chloroform extract	4+	4+	4+	4+	4+	4+
	Crude alkaloid extract	4+	4+	Nil	Nil	Nil	Nil
	Aqueous extract	4+	4+	Nil	Nil	Nil	Nil

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