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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTI-MITOTIC ACTIVITY OF ETHANOLIC EXTRACT OF FLOWERS OF NYCTANTHES *ARBOR-TRISTIS*

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Abstract:

The present paper deals with the Anti-Mitotic activity of Nyctanthes arbor-tristis [L], (NAT) is one of the sacred, traditional & mythological Indian medicinal plants in Ayurveda. The Methonolic extract of NAT flowers was evaluated for anti-mitotic activity. The In Vitro method for anti-mitotic activity is based on observation of sprouting of green gram (mung beans) was inhibited by NAT flower extract. The inhibition was found to be dose-dependent and is stable to quantify the anti-mitotic activity of NAT extract preparation. Cisplatin drug is used as standard. In future it will be interesting to evaluate the anti-mitotic activity of different plant parts.

Keywords: Nyctanthes arbor-tristis, Anti-mitotic activity, Ethanolic extract, Mung beans.

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INTRODUCTION:

There is a wide spread of demand for drugs derived from plants and people believe that medicines derived from plants are safe and dependable compared to synthetic drugs that have adverse effects. Mitotic inhibitors are derived from natural substances such as plant alkaloids, and prevent cells from undergoing mitosis by disrupting microtubule polymerisation, thus preventing cancerous growth. Nycanthes arbor-tristis is one of the plant that consists of alkaloids having anti mitotic activity .NAT is a night flowering sad tree of family Oleaceae (Nyctaginaceae) is well known in India .

PLANT DESCRIPTION:

NAT is usually a shrub or small tree having brilliant, highly fragrant flowers which bloom at night and fall off before sunrise, giving ground underneath a pleasing blend of white and red. Leaves are simple petiolate and exstipulate. Flowers are small delightfully fragrant sessile in pendaculate bracteates fascicles of 3-5, slender, hairy, auxiliary and solitary and in terminal short trichotomous chymes. Fruits are a capsule of 1-2 cm, long and broad, flat 1 seeded carpels reticularly veined, glabrous. Seeds are exalbuminous, testa thick.

PLANT TAXONOMY:

Kingdom	Plantae
Order	Lamiales
Division	Magnoliophyte
Class	Magnoliopsida
Family	Oleaceae
Genus	Nycanthes
Species	arbor-tristis



Fig 1: Nyctanthes arbor-tristis

Plant figures:



Fig 2: Plant figures

METHODS AND MATERIALS:

Collection of plant materials:

The flowers and leaves of Nyctanthes arbor-tristis [L] were collected in the month of January, February from local area which near to Guntur, Andhra pradesh, India. The plant specimen were identified and authentificated from Hindu college botanical garden.

Preparation of Extract

Apparatus Used: Maceration jar, Beaker, Test tubes stand, Petri-plates, Cotton cloth

Chemicals Used: Ethanol, Cisplatin drug, Water, NAT flower extract.

Solvent system: Ethanol

- **1.Extraction process:** Fresh flowers were collected and soaked in ethanol for 48 hrs. The extract was collected and concentrated by using Roravapourizer.
- **2.Phytochemical Screening:** The ethanolic extract was tested for the following natural products like carbohydrates, proteins, aminoacids, alkaloids, glycosides, steroids, saponins, and phenols by using different types of chemical tests. The results are tabulated in Table No.1

Materials required: Mung beans, Cis-Platin (STD), Plant extract with different concentrations (50μl, 100μl, 200μl), water

Determination of Anti-mitotic activity: Procedure:

rrocedure.

Mung beans used in this study were obtained from the local martket. Plant extract with with different volumes (500µl, 1000µl, 2000µl) were used and

mung beans of equal weight are weighed and soaked in each concentration respectively for 6 hours. Standard is prepared with same concentrations, with the anti-cancer agent Cisplatin 10mg. Control is prepared with mung beans soaked in tap water for 6 hrs. The water or the drug solution(test/std) was drained and trhe seedlings were kept moist (Either with tap water or the drug solution in a covered petri dish) until the radicals in the control group had grown to 1.0 to 3 cm (time 0,To) At To the weight of seedlings and length of radical were recorded both in control and test groups. The seedlings were maintained at room temperature under ,moist conditions for an additional period of 48 hrs.(T48). The weight of seedlings was measured again at T48. The percentage inhibition is calculated using the formula:

% Inhibition =
$$\frac{\text{(Wt D - Wt E)}}{\text{(Wt D - Wt STD)}}$$
 100

Where.

Wt D - wet weight of the seed in Distilled water; Wt E - wet weight of the seed in Extract Wt STD - wet weight of the seeds in Standard

RESULT AND DISCUSSION:

The present work deals with the study of flowers of NAT for their photochemical screening and antimitotic activity. Physicochemical studies were carried out for ethanolic extract by following qualitative tests. The results are tabulated in Table No.1. Based on that the ethaolic extract contains alkaloids along with other polar constituents like Glycosides, Steroids, Saponins, Flavonoids and Phenols. In Table No.2 "+" sign indicating positive result and "-"sign indicating negative result.

Table 1: Pl	ytochemical Screening	of ethanolic extract	t of flowers of NAT

TESTS	RESULTS
Steroids	- +
Flavonoids	- + +
Glycosides	- +
Phenols	+
Saponins	+
Alkaloids	+ +
Amino acids	-
Carbohydrates	-
Proteins	-

Table 2: IR-Bands of ethanolic extract of NAT

S.No	Functional group	IR- Range	IR-band
01.	Amine (N-H)	3300-3500	3307.07
02.	Alkane (C-H)	2850-2970	2951.07
03.	Aldehyde(-CHO)	2720-2850	2840.06
04.	Alkyne ($\underline{C} \equiv \underline{C}$)	2100-2260	2136.48
05.	Aromatic (C=C)	1600-1680	1647.09
06.	Amines(C-N)	1020-1360	1111.38

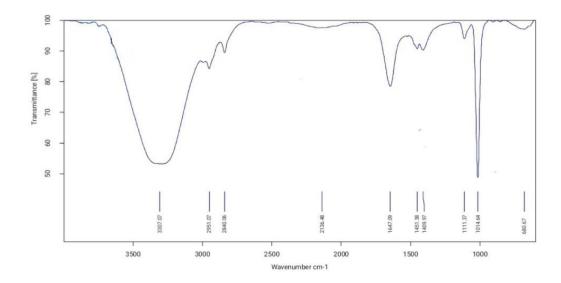


Fig 3: IR Spectrum of Ethanolic extract of flowers of NAT

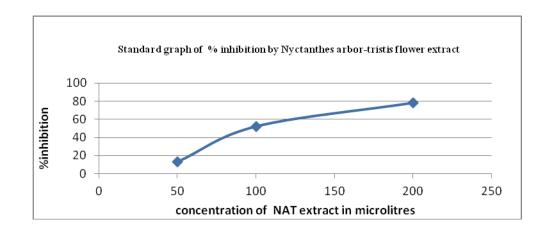


Fig 4: Standard graph of % inhibition by Nyctanthes arbor-tristis flower extract

The IR spectrum (Fig.3) of ethanolic extract of flowers of NAT shows that presence of different functional groups which are reported in the Table no.2

The anti mitotic activity of flower extract was studied by Percentage growth inhibition method by using Mung beans. The results are shown in Fig 2. The result from the study showed that the ethanolic extract of flowers of NAT had excellent Anti-mitotic activity that was compared to the activity of standard Cisplatin drug. The result was tabulated in the following Table.No.3.

Seeds that sprouted in presence of $500\mu l$ of extract had reduced growth with shortened shoot and at $2000\mu l$ of extract, there is inhibition of germination was complete with no visible sprouting. These morphological observations indicate dose dependence of inhibition.

Graph shows that the plant extract was exhibiting antimitotic activity dose dependant. The least concentration shows very less effect highest concentration shows potent effect

The growth of seed radicles are observed after 48hrs, the length of radicles was measured and values are in the TableNo.4.

Table 3: Report on percentage inhibition of growth

S.NO	DRUG OR	WET WEIGHT OF	WET WEIGHT OF	%INHIBITION
	EXTRACT	SEEDS AT T ₀ HRS	SEEDS AT T ₄₈ HRS	
		(In Grams)	(In Grams)	
1	CONTROL	1.03	2.07	
2	STANDARD	0.98	0.98	
3	GROUP-1	1.02	1.90	13%
4	GROUP-2	0.98	1.50	52%
5	GROUP-3	0.95	1.22	78%

Table 4: Average length of the seed radicles.

S.NO	SAMPLE	LENGTH OF RADICLE					AVERAGE	
		(In cen	(In centi meters)					LENGTH(In cm)
		1	2	3	4	5	6	
1	CONTROL	1.8	1.8	1.2	0.8	1.5	0.7	1.3
2	STANDARD	0.5	0.3	0.4	0.2	0.4	0.4	0.366
3	GROUP 1	0.8	0.9	0.2	0.1	0.2	0.1	0.3833
4	GROUP 2	0.6	0.7	0.3	0.2	0.3	0.1	0.3666
5	GROUP 3	0.4	0.5	0.2	0.3	0.1	0.1	0.266

Cisplatin is designed as an alkylating agent and acts specifically during DNA and RNA synthesis, and it is cytotoxic during the S-phase of the cell cycle. So, it was expected that the ethanolic extract of flowers of NAT showed its Anti-Mitotic activity same as that of standard (Cisplatin).

CONCLUSION:

In the present article, we have reported phytochemical screening and Anti-Mitotic activity of ethanolic extract of flowers of *Nyctanthes arbortristis*. The critical analysis of germination process on Mung beans has revealed that the plant exhibits an Anti-Mitotic activity dose dependent when compared with reference standard. We would like to extend our work in future to isolate the active ingredients present in the flowers.

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