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EVALUATION OF PHYTOCHEMICAL, ANIMICROBIAL AND CYTOTOXIC ACTIVITY OF *NYCTANTHES ARBORTRISTIS* METHANOLIC LEAF EXTRACT

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Abstract

Nyctanthes arbor-tristis Lin (family *Oleaceae*), which has been used traditionally for various medicinal purposes in the Indian subcontinent since long before. In view of this, the present study was designed to investigate for chemical constituents, anti-microbial and cytotoxic by methanolic extract of *Nyctanthes arbor-tristis.* Phytochemical screening revealed the presence of alkaloid, flavanoid, reducing sugar and steroid. In antimicrobial assay, the methanolic extract of the experimental plant was screened to antimicrobial activity against a wide range of both gram-positive and gram negative bacteria by disc diffusion method. The results obtained were compared with that of standard antibiotic, ciprofloxacin. In the brine shrimp lethality bioassay, the LC50 values of the sample was (0.11) µl/ml where the LC50 values of the standard potassium dichromate was (124.13) µl/ml as a positive control. The methanolic extracts are promising and further bioactivity-guided investigation can be done to find out potent antitumor and pesticidal compounds. This study shows that the methanolic extract of *Nyctanthes arbor-tristis leaves* has antimicrobial and cytotoxic activity.

Keywords: *Nyctanthes arbor-tristis, antimicrobial activity, cytotoxicity, analgesic activity, phytochemical evaluation*

Introduction

Plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally termed as medicinal plants. Since the down of civilization people are using plants and herbs to treat diseases. *Nyctanthes arbor-tristis* Linn is native in India and widely found in Bangladesh [1]. Different parts of *Nyctanthes arbor-tristis* Linn are known to possess various ailments by tribal the people of Indian subcontinent with its use in ayurvedic and unani medicines [2].The leaves of *Nyctanthes arbor-tristis* Linn are used extensively in Ayurvedic medicine for the treatment of various diseases such as sciatica, chronic fever, rheumatism, and internal worm infections, and as a laxative, diaphoretic and diuretic [3]. The leaves of this plants is used as stomachic, carminative, intestinal astringent, as expectorant, various skin diseases and as hair tonic [5]. It was evident that the leaves has antifungal activity as they have inhibitory effects on mycelial growth of dermatophytes and related keratinophilic fungi [5]. From literature review we observed that water soluble portion of the alcoholic extract of the leaves of this plant has central nervous system (CNS) activity. Worldwide, infectious disease is one of main causes of death accounting for approximately one half of all deaths in tropical countries. Our aim is to identify the antimicrobial activity of *Nyctanthes arbor-tristis* Linn leaves. Our study also include brineshrimp lithality bioassay. This bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, antiviral, pesticidal and antitumor etc. of the compounds. Brine Shrimp Lethality Bioassay technique stands superior to other cytotoxicity testing procedures because it is rapid in process, inexpensive and requires no special equipment or aseptic technique[6] . This study confirms the antimicrobial and cytotoxic activity of the *Nyctanthes arbor-tristis* although this plants is a miracle plant for the treatment of several diseases.

Methods

Leaves collection and extraction

The plant leaves was collected from Madaripur District month of November, 2017 at day time. After shade drying the leaves grinded and soaked with methanol for 15 days. The filtered liquid was extracted by using Rotary Evaporator [8].

Evaluation of Chemical group test

Testing of different chemical groups present in extract represents the preliminary phytochemical studies. In each test 10% (w/v) solution of extract in ethanol was taken unless otherwise mentioned in individual test. Though chemical Group Test is evident from the literature survey that the plant *Nyctanthes arbor-tristis* has some chemical groups. The work is an attempt to know about other chemical groups. Specific qualitative analysis were performed to identify the presence or absence of phytochemicals.

Microbiological Investigation

The in vitro antimicrobial study was designed to investigate the antibacterial spectrum of the crude extracts by observing the growth response. The rationale for these experiments is based on the fact that bacteria are responsible for many infectious diseases, and if the test materials inhibit bacterial growth then they may be used in those particular diseases [7]. The bacterial strains used for the experiment were collected as pure cultures from The Bangladesh Council of Scientific and Industrial Research (BCSIR) microbiology laboratory.

Evaluation of Cytotoxicity

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent cytotoxic activities, no side effects and economic viability. Brine shrimp eggs are hatched in simulated sea water to get nauplii. By the addition of calculated amount of Dimethyl sulfoxide (DMSO), desired concentrations of the test sample is prepared. The nauplii are counted by visual inspection and are taken in vials containing 5 ml of simulated sea water [9]. Then samples of different concentrations are added to pre-marked vials using micropipettes. Then the vials are left for 24 hour. Survivors are counted after 24 hours. These data are processed in Microsoft Excel for to estimate LC50 values.

Results

After phytochemical test it has been found that in the plant *Nyctanthes arbortristis* linn leaves contain

Flavonoids, Steroids, Tannins, Alkaloids and Glycosides. We found negative result for saponin test (Table 1).

In case of 1mg/ml of extract he maximum zone of inhibition found against Shigella dysentery as 10mm and no zone of inhibition found against Bacillus cereus. In case of 10mg/ml of extract the maximum zone of inhibition found against Staphylococcus aureus as 25mm and minimum zone of inhibition found against Salmonella typhi as 21mm. From the graph it is clearly seen that in case of 10mg/ml of extract the zone of inhibition found which is greater than the positive control (zone of inhibition by extract=22mm and the zone of inhibition by Azithromycin= 21mm both against Salmonella typhi) (Table 2).The lethal concentration (LC50) of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration and the best -fit line was obtained from the curve data by means of regression analysis. Potassium dichromate was used as positive control. The LC50 of the extract is 0.11µg/ml whereas the LC50 of the standard is 31.16µg/ml (Table 3; Figure 1 and 2). P<0.001.

Discussion

Phytochemical evaluation of the crude methanolic extract of the powdered plant material for the identification of the major group of phytochemicals. From literature we found that the leaves of Nyctanthes arbortristis contain D-mannitol, β-sitosterole, Flavanol glycosides-Astragaline, Nicotiflorin, Oleanolic acid, Nyctanthic acid, tannic acid, ascorbic acid, methyl salicylate, an amorphous glycoside, an amorphous resin, trace of volatile oil and so many chemical constituents. Our study indicates that leaves contain Alkaloids, Glycosides, Steroids, Gums, Reducing Sugarand Flavonoids[10],[1].Evaluation and screening of antibacterial activity of the crude methanolic extract of the powdered plant was done on both gram positive and gram negative bacteria[11][12].Many studies have demonstrated antiproliferative, cytostatic and cytotoxic activities[14] of phytochemicals against cancer cells. Our study demonstrated a significant response on cytotoxicity test.

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Table 1: Phytochemical test of N. arbostristis.

Chemical groups	Tests	Findings	Result
Alkaloids	Mayer's test	Yellow color precipitation	+
	Dragendroff's test	Orange brown color precipitation	+
Saponins	Foaming test	Foam was not considered	-
Glycosides	Sodium hydroxide test	Yellow color was present	+
	Fehling's test	No brick red precipitation	+
Steroids	Sulphuric acid test	Red color was formed	+
Gums	Molishc's test	Red violet ring was formed	+
Reducing Sugar test	Benedict's test	Red color precipitation formed	+
	Fehling's test	Red brick precipitation was present	+
Flavonoids	Hydrochloric acid test	Red color formed	+

+ =present and - =absent

Table 2: Zones of inhibition of methanolic extract of *Nyctanhes arbor-tristis* (leaves)

Microorganisms	ZMENA(1mg/ml)	ZMENA (10mg/ml)	Positive control Azithromycin (30 mcg/disk)
Vibrio mimicus	7 mm	17 mm	22 mm
Salmonella typhi	6 mm	22 mm	21 mm
Shigella dysentery	10 mm	17 mm	23 mm
Staphylococcus aureus	8 mm	18 mm	25 mm
Bacillus cereus	0 mm	14 mm	23 mm

N.B: ZMENA= zone of inhibition of methanolic extract of *Nyctanhes arbor-tristis*

Table 3: Data analysis of Cytotoxicity assay

Group	Concentration (C) µl/ml	Log C	Death	% of Death	LC_{50} µg/ml
Sample	400	2.6021	10	100	0.11
	200	2.301	10	100	
	100	2	10	100	
	50	1.699	10	100	
	25	1.398	9	90	
	12.5	1.097	8	80	
	6.25	0.7959	8	80	
	3.13	0.4955	7	70	
	1.56	0.1959	7	70	
	0.78	-0.108	6	60	
Standard	400	2.6021	7	70	31.16
	200	2.301	7	70	
	100	2	6	60	
	50	1.699	6	60	
	25	1.398	4	40	
	12.5	1.097	4	40	
	6.25	0.7959	4	40	
	3.13	0.4955	3	30	
	1.56	0.1959	2	20	
	0.78	-0.108	2	20	
Control	-	-	0	0	-

*Linear equation of sample: y = 15.90x + 65.15
**Linear equation standard: y = 20.33x + 19.62

Figure 1: % Mortality of Sample

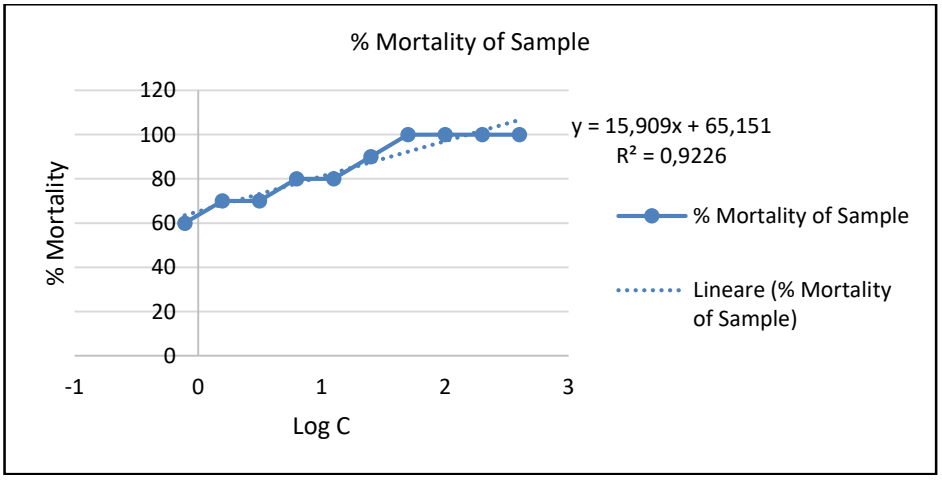

Figure 2: % of mortality of standard

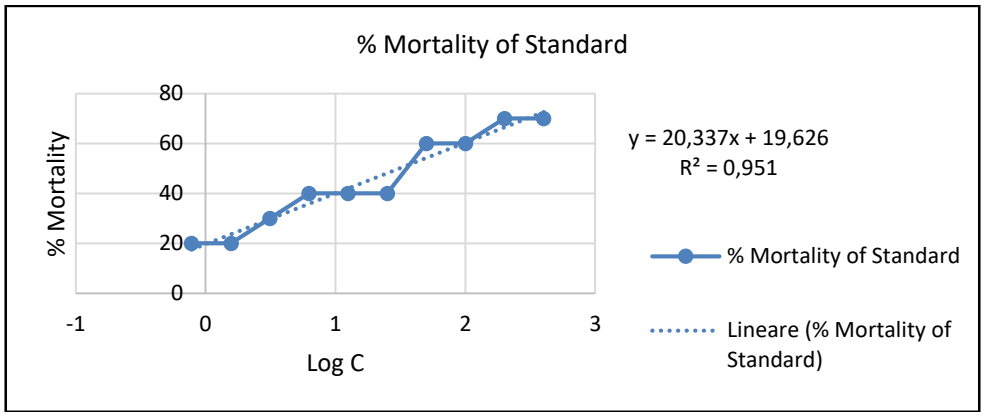

Figure 3: Comparison between cytotoxic effect of sample and standard