Original Research

PHYTOCHEMICAL SCREENING AND CYTOTOXIC ACTIVITY OF *NYCTANTHES ARBOR-TRISTIS*

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ABSTRACT

Objectives: The present study aims at studying phytochemical screening and cytotoxic activity of ethanolic extracts of *N.arbor-tristis* by performing brine shrimp bioassay.

Materials and methods: The aerial stem and leaves were subjected first hexane extraction to remove fats then subjected to the ethanol extraction using soxhlet method. The extract was subjected to qualitative and quantitave phytochemical screening and cytotoxic activity by performing brine shrimp bioassay.

Results: The phytochemical screening of the plant was found to exhibit the positive reaction test for flavonoid, phenolic compound, tannin, terpenoids, saponin, alkaloids and reducing sugar. The brine shrimp bioassay revealed that ethanol extracts of the plants showed the cytotoxic activity. The alkaloids and tannins present in the ethanol extract might be responsible for cytotoxic activity.

Conclusions: The study revealed that the plant possessed various chemical constituents from which lead molecule can be generating for development of newer drugs. The brine shrimp bioassay of the ethanolic extract of the plant showed that the plant possessed the Cytotoxic activity as well However, to reach conclusive decision detail study for characterization, isolation, purification and identification of compound and biological studies with exact mechanism of action responsible for the Cytotoxic activity is necessary.

KEY-WORDS: Brine-shrimp bioassay, Cytotoxic, Ethanolic extract, *N. arbor-tristis,* Phytochemical screening

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INTRODUCTION

Herbs have been always the main principle form of medicine since traditions and now a days it becoming more popular form of medicine throughout the world. Herbal medicines are not only providing traditional and ethnic medicine but also promising for highly efficient novel bioactive molecules. Medicinal plants are a reservoir of various chemical compounds which serve as drugs and the potential source for newer lead molecule and clues for modern drug design by synthesis [1].

Nyctanthes arbor-tristis Linn is one of the well known medicinal plants. It is a common wild hardy large shrub or small tree. It is native of India, distributed wild in sub-Himalayan regions and southwards to Godavari, Lalitpur Nepal. *Nyctanthes arbor-tristis* Linn is commonly known as Night Jasmine or Parijata· *Nyctanthes arbor-tritis* is a large shrub growing to 10 m tall, with young branches and rough leaves. The flowers are fragrant, with a five- to eight-lobed white corolla with an orange-red centre. Calyx is 6-8 mm long, narrowly campanulate, hairy outside, glaborous inside truncate or obscurely toothed or lobed, ciliated. The leaves are opposite, simple, 6–12 cm long and 2–6.5 cm broad, with an entire margin. The fruit is a flat brown heart-shaped to round capsule 2 cm diameter, with two sections each containing a single seed [2].

Different parts of this plant are used in Indian systems of medicine for various pharmacological actions like cytotoxicity [3], anti-inflammatory, antidiabetic, hepatoprotective[4], antioxidant and antimicrobial activities [5]. Various research works reveal that the plant contains various chemical constituents like alkaloids, glycosides, terpenoids, tannins etc [6]. But the constituents may vary according to the altitude the plant is located. The present study aims at studying phytochemical screening and cytotoxic activity of ethanolic extracts of *N.arbor-tristis* by performing brine shrimp bioassay.

MATERIALS AND METHODS

The aerial part including stems and leaves of the plant were collected from Dhapakhel, Thecho height, Lalitpur, Nepal and then it was identified as *Nycthanthes arbor-tritis* in National Herbarium, Godawari, Nepal.

Preparation of Plant Extract

The aerial parts of the plant was dried at room temperature and then crushed into cutter mill as to make a coarse powder. The powder was then successively extracted in Soxhlet apparatus using hexane and ethanol respectively. The extracts were then dried in water bath at around 70°C.

Phytochemical Screening

The phytochemical screening (qualitative) was done to identify the main groups of chemical constituents glycosides, alkaloids, saponins, terpenoids, steroids, flavonoids, phenolic content, reducing sugars and tannins present in ethanolic extract of *Nyctanthes arbor-tristis* by their color reactions with different reagents. The extract was also subjected to quantative screening for alkaloids, saponins, flavonoids, phenolic content and tannins [7,8,9].

Brine Shrimp Bioassay for Cytotoxic study

Brine shrimp bioassay is a rapid, reliable, inexpensive and convenient method in which natural

product extracts, fractions or pure compounds are tested [10]. It is one of the best and rapid method especially with plant extracts [11]. It is found that ED_{50} values of general cytoxicities are about one-tenth LC_{50} value in brine shrimp test [10].

The toxic levels from brine shrimp bioassay are as follows: [12]

LC_{50} Value	**Remark**
<1.0µg/ml	Highly Toxic
1-10.0µg/ml	Toxic
10.0-30.0µg.ml	Moderately Toxic
30-100µg/ml	Mildly Toxic
>100µg/ml	Non-Toxic

The Cytotoxic activity of the ethanolic extract of the plant was carried out as per the method described by **[13].**

Cytotoxicity test depends on the calculation of LC_{50} value [14]. The LC_{50} value for the given extract is the lethal concentration that is required to kill the 50% of the brine shrimp nauplli. LC_{50} value can be calculated as follows:

If 'n'= the number of replicates (here three), 'x'=the log of concentration in mg/ml (here log50, log125, log250, log500, log1000 for three doses levels respectively) and 'y'= probit value (i.e., average survival of all the replicates)

We have,

$$\alpha = \frac{1}{n}\left[\sum y - \beta \sum x\right]$$

Where,

$$\beta = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sum x2 - \frac{(\sum x)2}{n}}$$

From Probit regression,

$$Y = \alpha + \beta X$$

Where,

$$X = \frac{Y - \alpha}{\beta}$$

Where, Y is constant having value 5 for calculating LC_{50}-value.

Thus, the LC_{50}-value can be given as,

$$LC_{50} = \text{Anti log } X$$

With the help of the above expression, the LC_{50} values for different extract can be determined.

Statistical Analysis of the data

Data obtained from experiments were expressed as mean ± S.E.M/ S.D using SPSS version 17.0, MS Excel 2007. Statistical differences were evaluated by paired t test, Analysis of Variance (ANOVA) and Post hoc Tukey tests, Benferroni test and p value of < 0.05 was considered to be significant. The data were expressed as mean [95% confidence interval for mean].

RESULTS AND DISCUSSION

Phytochemical Screening

Qualitative method of phytochemical screening

Phytochemical Screening of the plant showed the presence of different group of active constituents in ethanol extracts. The different chemical constituents in the ethanolic extract are given in table 1.

Table No. 1: Phytochemical screening of stem and leaves of *N. arbor-tristis*.

S.N	Test Compound	Result with EE
1	Alkaloids	+
2	Fehling's test for carbohydrate	+
3	Glycosides	
	Legal's test for glycosides	-
	Borntrager's test for glycosides	-
4	Phenolic compound and tannin	+
5	Terpenoids	+
6	Proteins and amino acids	
	Ninhydrine	-
7	Saponin	+
8	Flavanoids	+
9	Reducing Sugar	+

Absent (-) and Present (+), EE: Ethanolic extract

Quantitative method of phytochemical screening

Determination of total tannin

The absorbance of test solution of different concentrations and their respective absorbance have been tabulated in table 2. Then a standard calibration curve was prepared to quantify the total tannin present in the ethanolic extract. The standard calibration curve is shown in figure 1.

Table No. 2: total tannin content

SN	Stock Solution(ml)	Folin-ciocalteu's phenol reagent(ml)	Saturated Na_2CO_3 Solution (ml)	DW(ml) up to	Conc. (mg /ml)	Abs (760 nm)
1	1	5	10	100	0.01	0.152
2	2	5	10	100	0.02	0.238
3	3	5	10	100	0.03	0.332
4	4	5	10	100	0.04	0.444
5	5	5	10	100	0.05	0.531
6	Blank	5	10	100	0	0

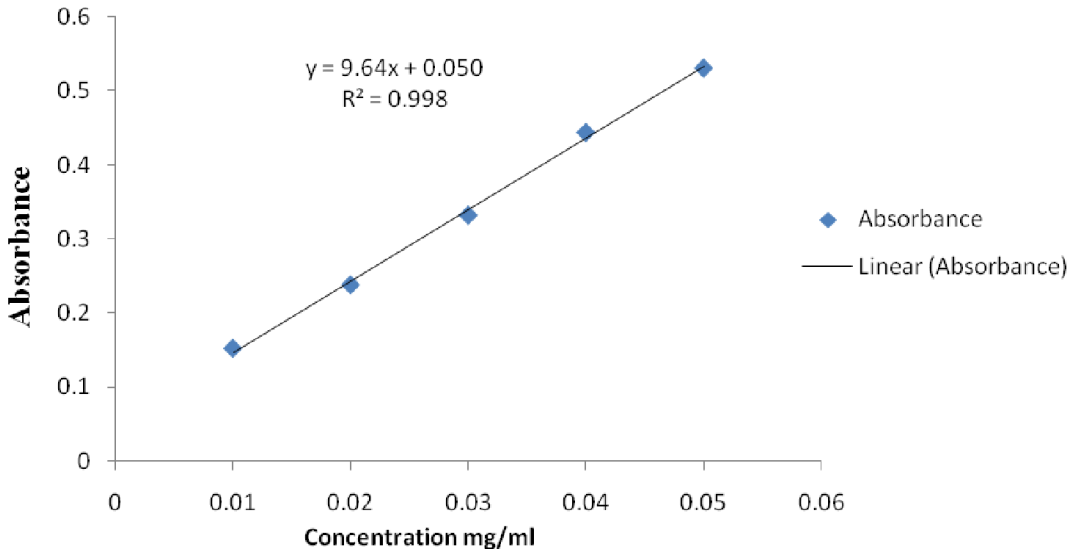

Fig No. 1: Calibration curve for total tannin content

The concentration of tannin is shown in table 3.

Table No. 3: Concentration of tannin mg/g

Absorbance	Concentration	Mean ±SD
0.302	2.96148	
0.303	2.97112	
0.312	3.05788	3.06174±0.09654
0.322	3.15428	
0.323	3.16392	

Determination of total flavonoids

The absorbance of test solution of different concentrations and their respective absorbance have been tabulated in table 4. Then a standard calibration curve was prepared to quantify the total flavonoids present in the ethanolic extract. The standard calibration curve is shown in figure 2.

Table No. 4: Total flavonoids content

SN	Stock (ml)	2% $AlCl_3$ solution(ml)	DW (ml) up to	Conc. mg/ml)	Abs(420 nm)
1.	0.2	0.5	10	0.002	0.411
2.	0.4	0.5	10	0.004	0.749
3.	0.6	0.5	10	0.006	1.165
4.	0.8	0.5	10	0.008	1.314
5.	1	0.5	10	0.010	1.740
6.	Blank	0.5	10	0	0

The concentration of flavonoids is shown in table 5.

Table No. 5: Concentration of flavanoid in mg/g

Absorbance	Concentration	Mean±SD
0.054	0.195921	
0.065	0.2136475	
0.058	0.202367	0.20301±0.0067
0.059	0.2039785	
0.056	0.199144	

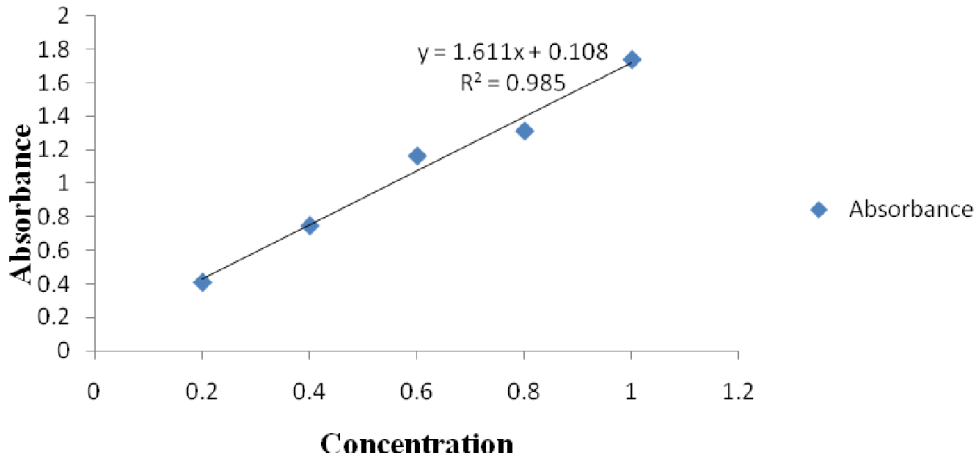

Fig No. 2: Calibration curve for total flavanoid

Determination of total phenolics

The absorbance of test solution of different concentrations and their respective absorbance have been tabulated in table 6. Then a standard calibration curve was prepared to quantify the total flavonoids present in the ethanolic extract. The standard calibration curve is shown in figure 3.

Table No. 6 : Total phenolic content

SN	Stock Solution(ml)	Folin-ciocalteu's phenol reagent(ml)	Saturated 20%Na_2CO_3solution (ml)	DW(ml) Upto	Conc. (mg /ml)	Abs (760 nm)
1	1	1.5	4	25	0.04	0.248
2	2	1.5	4	25	0.08	0.341
3	3	1.5	4	25	0.12	0.434
4	4	1.5	4	25	0.16	0.556
5	5	1.5	4	25	0.20	0.645
6	Blank	1.5	4	25	0	0

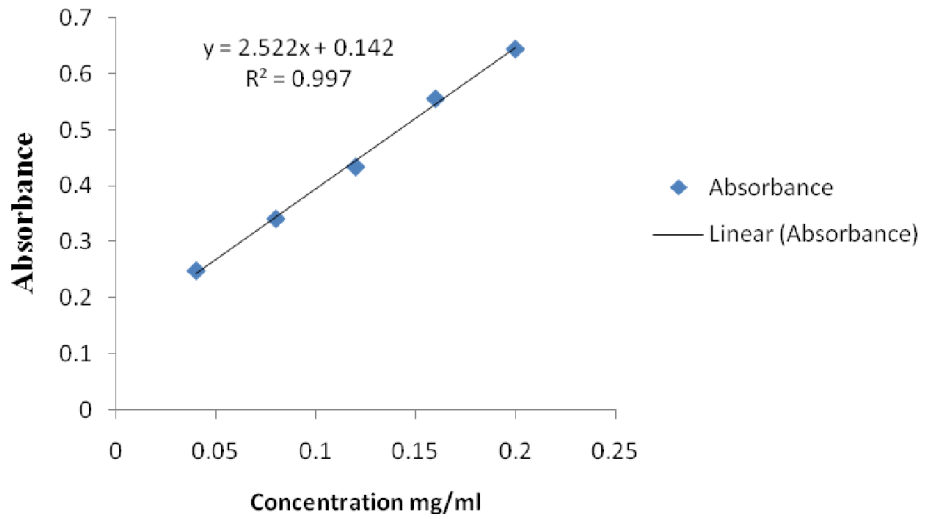

Fig No. 3: Calibration curve of total phenolic content

Total phenolic content in the test sample is shown in table 7.

Table No. 7: Concentration of Total phenolic content in mg/g

Absorbance	Concentration	Mean±SD
0.756	2.04911	
0.678	1.852355	
0.778	2.104605	2.10006±0.18518
0.883	2.3694675	
0.786	2.124785	

Determination of alkaloids and saponin

The weight taken of alkaloid W_1 = 5.03 g

The weight taken of saponon W_1 = 5.08

Weight of residue of alkaloids W_2 = 0.2 g

Weight of residue of alkaloids W_2 = 0.5 g

% of alkaloids = 397.61 mg

% of alkaloids = 984.2 mg

Nyctanthes arbor-tristis contain chemical constituents like polysaccharides, iridoid glycosides, phenylpropanoid glycoside (nyctoside A), ß-sitosterol, ß-amyrin, hentri-acontane, benzoic acid, glycosides, nyctanthoside-a iridoid, nyctanthic acid, Friedelin and lupeol and oleanolic acid and 6ß-hydroxylonganin [15], and iridoid glucosidesarborsides A, B and C, alkaloids, Phlobatanins, terpenoids and cardiac glycosides. Iridoid glucosides (arbortristosides-A, B, C) and 6ßhydroxyloganin, 4-hydroxy hexahydrobenzofuran-7-one tertiary alkaloids mainly 7-(alpha-anilino-p-nitrobenzyl)-8-quinolinol and quarternary alkaloids belonging to protoberberines , and aporphines [16] , has also been isolated from this plant.

The preliminary phytochemical screening of the plant was found to exhibit the positive reaction test for flavonoid, phenolic compound, tannin, terpenoids, saponin, alkaloids and reducing sugar. The result is in consistent with the findings by Sathiya M et al. which showed that the ethanolic extract of leaves contained alkaloids, tannins, flavonoids and cardiac glycosides [17].

In Chloroform and ethyl acetate extracts of leave, fruits and seed shows the presence of phytosterols. Phenolics compounds, tannins, flavonoids, cardiac glycosides, saponins and alkaloids content. Steroids were present only in seeds [6].

Sah, A.K. and Verma review the article and various parts of plant like seeds, leaves, flowers, bark and fruits have been investigated for their significant phytochemicals. Phytochemicals like flavanol glycoside, oleanic acid, essential oils, tannic acid, carotene, friedeline, lupeol, glucose, benzoic acid have been reported.[1]

A study on preliminary phytochemical screening of ethanolic extract of *N. arbor-tristis* showed that the presence of alkaloids, tannins, terpenoids, falvanoids, and cardia glycosides[17]. Similar type study in chloroform and ethyl acetate extract showed the presence of phenolic compound, tannins, flavanoids, cardiac glycosides, saponin and alkaloid content [6]. This indicates the insolubility of certain group of compounds in certain solvents. Difference of solubility of the compounds in different solvents can be used in the separation of the compounds presents in the plant. Thus phytochemical screening helped in identifying the main chemical constituents present in the extract.

Brine Shrimp Bioassay Activity

Ethanol Extract of *Nycthanthes arbor-tritis* showed cytotoxic activity which has LC_{50} value of 193.1716µg/ml. The result for each ethanol extract is given in table 8.

Table No.8: Effect of ethanol extract on Brine Shrimp when y = Average no. of survival

Plant extract	**Concentration (z) (ppm)**	**Log(z) = x**	**Avg. no. of survival (y)**	**LC 50 (µg/ml) [antilog X]**
	100	1.698	7.66	
	300	2.096	6.33	193.1716
Extract	500	2.397	5.66	
	1000	2.699	2.66	
	2000	3.000	0.00	

The % mortality of brine shrimp at different concentration of ethanolic extract is given in table 9 and the regression equation of % mortality of brine shrimp nauplii Vs Log (conc.) of ethanolic Extract.

Table No. 9: Mortality of Brine shrimp at different concentration of ethanol extract.

Extract Concentration	Mortality± SEM	% Mortality
50µg/ml	2.33±0.6667	23.33
125µg/ml	3.33±0.6667	36.67
250µg/ml	4.667±0.8819	46.67
500µg/ml	7.333±0.3333	73.33
1000µg/ml	10.00±0.0000	100

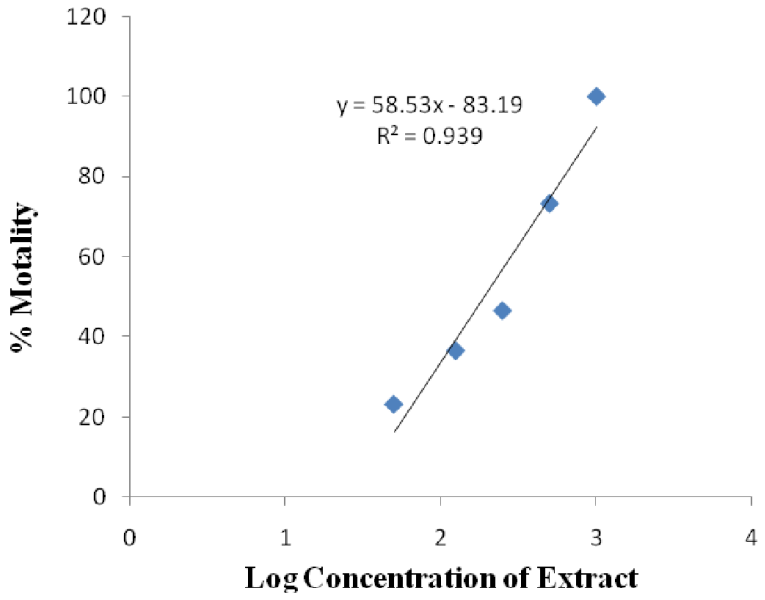

Fig No. 4: Regression equation of % mortality of brine shrimp nauplii Vs Log (conc.) of ethanolic Extract.

The brine shrimp lethality bioassay revealed that ethanol extracts of the plants showed the cytotoxic activity. The cytotoxicity bioassay against *Artimia salina* is a simple and inexpensive method to test cytotoxicity, to biodirect phytochemical fractionation of natural products and a predictor of antitumor and pesticidal activity [18]. Brine shrimp lethality is the bioassay useful for screening large number of extracts in the drug discovery process. The LC_{50} values of the brine shrimp obtained for ethanol extract of *N. arbor-tristis* have been found as 193.1716µg/ml. The degree of lethality was found to

be directly proportional to the concentration of the extract. In the evaluation for general toxicity using brine shrimp, maximum mortalities took place at a concentration of 1000 µg/ml whereas; least mortalities were at 50 µg/ml concentration. The LC50 values of the plant extracts (24 hrs) were obtained by a plot of percentage of the shrimps killed against the concentrations of the extracts and the best-fit line was obtained from the data by means of regression analysis. The alkaloids and tannins present in the ethanol extract might be responsible for cytotoxic activity [20]. However a detail studies in the constituent of *N. arbor-tritis* is required to identify the active constituent(s) responsible for the activities.

CONCLUSION

It was found that the plant possessed different phytochemical constituents like phenolic compounds, tannins, flavanoids, alkaloids, glycosides, reducing sugar, terpenoids and saponin. The brine shrimp bioassay of the ethanolic extract of the plant showed that the plant possessed the Cytotoxic activity as well. It may, therefore, be concluded that the plant possessed different chemical constitutents which may generate the lead molecules for development of newer drugs. It is recommended that further phytochemical and pharmacological studies to identify the principle constituents responsible for the cytotoxic activity be carried out for better evaluation of the potential effectiveness of the plant, so that its chemicals or phytochemicals can be utilized as an alternative medicine in future.

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