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Original Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND TO EVALUATE THE ANTI-MICROBIAL ACTIVITY OF HYDRO-ALCOHOLIC and PETROLEUM ETHER EXTRACT OF JASMINE ROOT (*NYCTANTHES ARBOUR-TRISTIS*). (FAMILY-NYCTAGINACEAE)

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ABSTRACT

Objective: To estimate the anti-microbial activity of hydro-alcoholic (methanol) and petroleum ether extract of *Nyctanthes arbour-tristis* (family-Nyctaginaceae) in conjugation with phytochemical screening.

Methods: The hydro-alcoholic and petroleum ether extract of the whole root part of the plant *Nyctanthes arbour-tristis* (family-Nyctaginaceae) was prepared and studied for phytochemical constituents by using various standard methods. The antimicrobial activity of plant extract was performed on two bacterial strains and one fungal strain using disc diffusion method.

Results: The present study shows the phytochemical analysis, antimicrobial activity of the hydro-alcoholic and petroleum ether extract of the root of *Nyctanthes arbour-tristis*. Various phytochemical analyses revealed the presence of alkaloids, carbohydrates, flavonoids, tannin, phenol, terpenoids, glycosides, saponins respectively. The anti-microbial activity of the plant extract showed significant results against all three of the test organisms.

Conclusion: The present study concluded that the hydro-alcoholic and petroleum ether extract of the root of *Nyctanthes arbour-tristis* (night flowering jasmine) contains the highly presence of Phytochemical constituents. The hydro-alcoholic and petroleum ether extract of the plant was found to possess promising antimicrobial activity when compared with the standards.

Keywords: *Nyctanthes arbour-tristis,* Antimicrobial activity, Disc diffusion method, Zone of inhibition, Phytochemical analysis

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INTRODUCTION

Nyctanthes arbortristis is an important and useful plant for its various activity and for its medicinal use as well as cultural use, *Nyctanthes arbortristis* plant is also known by 'night flowering jasmine' which belong to family oleaceae but now it has been changed and named as nyctaginaceae. This plant mainly developed and growth in the tropical and subtropical area, the place like India, central Australia, USA etc. The name night flowering jasmine is given because of the flowers are seen during night and fall after the mid night. Nyctanthes word is taken from a Greek word that is nyktha and anthos. Whereas nyktha mean night and anthos mean flower.

Nyctanthes arbortristis having a various therapeutic activity like anti-fungal, anti-pyretic, anti-histaminic, anti-oxidant, anti-inflammatory and much more which going to discover and identify. The aim of using the herbal drug is to minimise the side effect with better efficacy [1-3].

MATERIALS AND METHODS

Collection of plant materials

The fresh *Nyctanthes arbortristis* (night flowering jasmine) was collected from Azara, Guwahati, Assam, India. The plant was authenticated by Dr. P. P. Baruah, professor and head, Department of Botany, Gauhati University, Guwahati, Assam, India. The specimen stored by the department of botany, Gauhati University with Acc. No.-18381 dated 23-11-2017 and the reference number is Herb./Bot./GU/2017/166.

Chemicals and reagents

Petroleum Ether, Methanol, Dragondorff reagent, Mayer's reagent, Wagner's reagent, Benedict's reagent, sulphuric acid, lead acetate, Molisch's reagent, Fehling solution A and B, sodium citrate, copper sulphate, ferric chloride, sodium hydroxide, glacial acetic acid, benzene, chloroform, ammonia, nitric acid, potassium nitrite, gelatine, Beef extract, Peptone, distilled water, agar etc.

Preparation of the plant extract

The root of night flowering jasmine was collected and washed with tap water, remove all the soil and dirt's and shade dried. Shade-dried roots were grinded and formed powdered, then it passed through sieve number 60 and then the material was extracted with non-polar to polar solvents. At first, the plant material was defatted with petroleum ether then the extraction is carried out in a hydro-alcoholic solvent that is methanol and distilled water in a Soxhlet apparatus by continuous heat extraction. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50 °C [4].

Phytochemical screening

Alkaloids

Reagent: Mayer's test (Potassium mercuric-iodide solution)

Procedure: Filtrates were treated with Mayer's reagent

Result: Yellow coloured precipitate formation indicates the presence of alkaloids.

Carbohydrate

Reagent: Molisch's Test (α-naphthol solution)

Procedure: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube

Result: Violet ring formation at the junction indicates the presence of carbohydrates.

Flavonoids

Reagent: Lead acetate Test (lead acetate solution)

Procedure: Plant extract was treated with few drops of lead acetate solution.

Result: Yellow colour precipitate formation of indicates the presence of flavonoids.

Tannins

Reagent: Gelatin Test (Sodium Chloride)

Procedure: To the plant extract, 1% gelatin solution containing sodium chloride was added.

Result: Buff colour precipitate formation indicates the presence of tannins.

Phenols

Reagent: Ferric chloride solution.

Procedure: Plant extract was treated with ethyl acetate.

Result: Formation of green or violet colour indicates the presence of phenol.

Protein

Reagent: Xanthoproteic Test (conc. nitric acid)

Procedure: Plant extract was treated with few drops of conc. nitric acid

Result: Yellow colour formation indicates the presence of proteins.

Terpenoid

Reagent: Salkowski test (conc. sulphuric acid)

Procedure: Few drops of extract treated with few drops of conc. Sulphuric acid, shake it nicely and allowed to stand for some time.

Result: Formation of yellow coloured at lower layer indicated the presence of terpenoids.

Glycoside

Reagent: Pyridine, sodium nitroprusside, Sodium hydrooxide solution

Procedure: Plant extract are treated with pyridine and add sodium nitroprusside solution.

Result: Blood red colour appears to indicate the presence of glycoside.

Saponin

Reagent: Foam test (Sodium bicarbonate)

Procedure: Place drug solution in water in a test tube and shake well.

Result: Froat foam is formed [5-7].

Table 1: Microorganisms used for anti-microbial activity

Bacterial strain				**Fungal strain**	
Gram positive organism	ATCC No.	Gram negative organism	ATCC No.	Name	ATCC No.
Staphylococcus aureus	ATCC3127	*Escherichia coli*	ATCC1724	*Candida albicans*	ATCC2429

Microbial strains

The strain was preserved and kept under fully sterile conditions and grown on Nutrient Agar media for bacteria *Staphylococcus aureus* and *Escherichia coli,* Sabourand dextrose agar media for fungi *Candida albicans* in the Microbiology Laboratory of Girijananda Chowdhury Institute of Pharmaceutical Science, Guwahati, India.

Preparation of extract solution

The hydro-alcoholic and Petroleum ether extract were prepared by dissolving 0.3 gm in 0.1 ml that is 100 µl of 100 percent DMSO and q. s to 1 ml that is 1000 µl with distilled water and stored under refrigerator condition, and then different concentration was prepared according to requirements.

Anti-microbial activity

Anti-bacterial assay

Plant extract of concentration 25, 50, 100 µl respectively was prepared on the day of experiment after that agar media was placed in the Petri plate and placed in refrigerator for solidifying purpose, after some time it was then taken out and inoculums of each test organism was spread onto the agar plate, so as to achieved a confluent growth different concentration of extract was introduced into the Petri plate. The plate was then incubated at 37 °C for 24 h, after that zone of inhibition was observed and measured [8].

Anti-fungal assay

The dextrose agar plate was prepared and inoculated with *Candida albicans*. The zone of inhibition was measured in millimetres (mm) after 24hr incubation and compared with the standard antifungal drug (ketoconazole) which was then used as positive control and DMSO 10 percent as a negative control.

RESULTS

The study shows the phytochemical screening, anti-microbial activity of the hydro-alcoholic and petroleum ether extract of the plant *Nyctanthes arbortristis.* The yield % of the extraction of the hydro-alcoholic was 3.65 % and petroleum ether 3.88%.

Phytochemical screening

Table number 2 showed the result of Phytochemical screening of both extract hydro-alcoholic and petroleum ether. Where the (+) positive mean present and (-) negative mean absent.

Anti-microbial activity

Table number 3 showed the antimicrobial activity of the plant extract. The hydro-alcoholic and petroleum ether extract of the *Nyctanthes arbortristis* root having anti-microbial activity against both gram positive and gram negative bacteria and fungi.

Table 2: Phytochemical screening of hydro-alcoholic and petroleum ether extract of root of *Nyctanthes arbortristis*

S. No.	**Test for constituents**	**Petroleum ether**	**Hydro-alcoholic(methanol)**
1	Alkaloids	+	-
2	Carbohydrates	-	+
3	Flavanoids	-	+
4	Tannin	-	+
5	Phenol	-	+
6	Protein	-	-
7	Terpenoids	-	+
8	Glycosides	-	+
9	Saponins	+	+

(-) Absence, (+) Presence

Table 3: Antimicrobial activity of root hydro-alcoholic and petroleum ether extract *Nyctanthes arbortristis* observed against the growth of some plant pathogenic bacteria using disc diffusion method

Name of the compounds with concentration	Anti-bacterial activity diameter anti-fungal activity diameter of		
	Zone of inhibition (mm)	Zone of inhibition (mm)	
	Escherichia coli	*Staphyllococcus aureus*	*Candida albicans*
Chloramphenicol (30 µl)	31.26	28.55	----
Ketoconazole (10 µl)	----	----	22.27
Hydro-alcoholic (25 µl)	17.65	13.14	12.34
Hydro-alcoholic (50 µl)	20.10	16.22	14.24
Hydro-alcoholic (100 µl)	22.58	18.77	17.19
Petroleum ether (25 µl)	10.18	8.87	7.12
Petroleum ether (50 µl)	12.08	10.11	9.57
Petroleum ether (100 µl)	13.89 12.26 11.57		

Zone including 5 mm of paper diameter

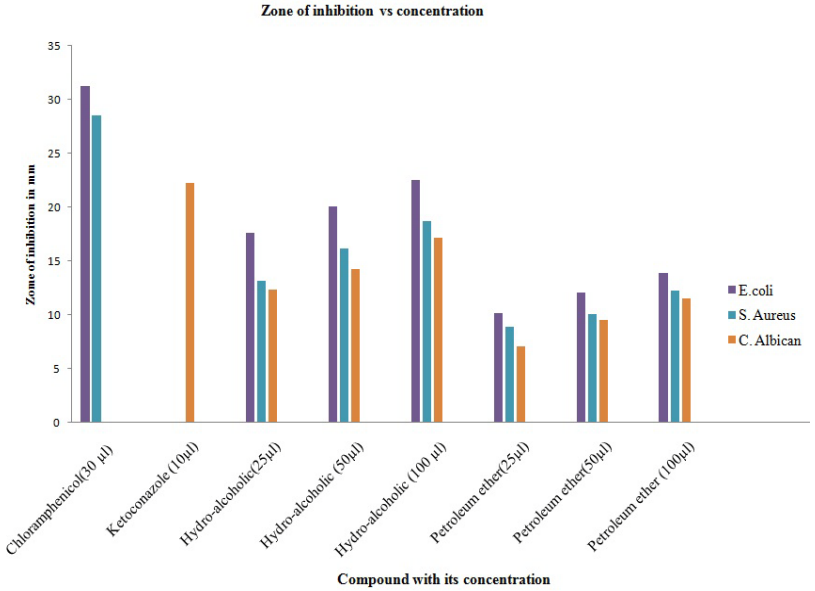

Fig. 1: Zone of inhibition vs concentration

DISCUSSION

Phytochemical analysis

The Phytochemical test of *Nyctanthes arbortristis* root showed the presence of various phytoconstituents. Hydro-alcoholic extract having Carbohydrates, Flavanoids, Tannin, Phenol, Terpenoids, Glycosides, Saponins and petroleum ether extract having Alkaloids, Saponins (table 2).

Anti-bacterial and anti-fungal activity

The anti-microbial activity of the plant extract of *Nyctanthes arbortristis* is done by the agar disc diffusion method. Chloramphenicol and Ketoconazole drug taken as the standard for anti-bacterial and anti-fungal activity and it showed the potent activity and the result is given in table 2.

In case of bacteria, both extract at concentration 25 µl shown the minimum zone of inhibition whereas 100 µl shown the maximum zone of inhibition against the bacterial strain of *Escherichia coli* and *Staphyllococcus aureus.*

In case of fungi, both extract at concentration 25 µl shown the minimum zone of inhibition whereas 100 µl shown the maximum zone of inhibition against the fungal strain of *Candida albicans.*

CONCLUSION

The hydro-alcoholic and petroleum ether extract of *Nyctanthes arbortristis* (night flowering jasmine) part root confirms it having antimicrobial property. The antimicrobial activity was evaluated by disk diffusion method, in this study the micro-organisms were taken *Staphyllococcus aureus* (gram+ve bacteria), *Escherichia coli* (gram–ve bacteria), *Candida albicans* (fungi).

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declare none

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