PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF NYCTANTHES ABBOUR-TRITIS

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ABSTRACT

The present study was undertaken to investigate the phytochemical and antimicrobial analysis of *Nyctanthes arbor-tristis*. *Nyctanthes arbor-tristis* contains phenolic compounds, glycosides, carbohydrates, proteins, saponins and alkaloids. Triterpenoids, tannins and anthraquinone were absent. Antimicrobial activity of methanolic extract showed that *Escherichia coli* and *Staphylococcus aureus* were highly sensitive.

Key words: Agar well diffusion, *Escherichia coli*, *Nyctanthes arbor-tristis*, Phytochemicals.

INTRODUCTION

Nyctanthes arbour-tritis is commonly known as night flowering jasmine (Parijat). It is native to Southern Asia from Pakistan, India, Nepal and Thailand. Its seeds, leaves and flowers are useful as medicine. They are used for chronic fever, bronchitis, asthma, constipation, greyness of hair, skin diseases, sciatica, rheumatism and baldness. The seeds of *N. arbor-tristis* are used in treatment of piles. From its leaves three new benzoic esters of Loganin and 6- β-hydroxyloganin, namely arborside-A, arborsideB, and arborside-C were isolated. Leaves also contain the alkaloid nyctanthine along with nannitol, βAmyrin β-Sitosterol, hentriacontane, benzoic acid, astragalin, nicotiflorin, oleanolicacid, nyctanthic acid, friedelin and lupeol[1, 2]. Many research groups have also reported such studies throughout the world [3, 4]. Phytochemicals previously with known pharmaceutical activities have been investigated as a source of medicinal agents [5]. It is well known that phytochemicals with adequate antibacterial and antifungal efficacy will be used for the treatment of bacterial and fungal infections [6]. Yadav et al., 2018 and Yadav, 2018 tested the antimicrobial activity of plants and showed that plants are a potential source of innovative antibiotic prototype [7, 8]. The present study was undertaken to investigate the phytochemical and antimicrobial analysis of Nyctanthes arbor-tristis and secondary metabolite present in it.

MATERIALS AND METHODS

Preparation of Plant Extract

Leaves of plant were collected from garden and were washed under tap water and distilled water. 10 g of powder was used for solvent extraction via Soxhlet apparatus with methanol. The extract was evaporated at room temperature.

Phytochemical screening

Phytochemical screening was carried out to determine the presence of saponins, tannins, flavonoids, glycosides, terpenoids, phytosterols and cardiac glycosides, proteins, carbohydrates and phenols.

1. Test for Saponins (Foam test)

To test presence of saponins 200 mg of sample powder was mixed with 5 ml of distilled water and was shaken vigorously for a stable persistent broth. Foam formation confirmed the presence of saponins in extract.

2. Test for Tannins (Ferric chloride test)

Few drops of 0.1% ferric chloride solution was added in extract. Formation of blue black colour indicated the presence of tannins in extract.

3. Test for Alkaloids (Wagner's test)

2-3 drops of Wagner's reagent was added in 0.5ml extract. Formation of reddish brown precipitate indicated the presence of alkaloids.

4. Test for Flavonoids (Alkaline reagent test)

Few drops of sodium hydroxide were added in the extract solution. First formation of an intense yellow color and then turning in to colourless solution on addition of few drops of dilute acetic acid indicate the presence of flavanoids in extract.

5. Test for Sterols and Triterpenoids (Salkolwski's test)

After treating with chloroform, few drops of concentrated H_2SO_4 was added in extract, the test tube will be shaken well and allowed to stand for some time. The appearance of red colour in upper layer confirmed the presence of sterol and formation of yellow colour at the lower layer confirmed the presence of triterpenoids in extract.

6. Test for Glycosides (Keller Killani test)

After drying with chloroform, 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride solution was added in extract. 0.5 ml of concentrated H_2SO_4 was added along the sides of the test tube. Blue color formation in acetic acid layer indicated the presence of cardiac glycosides in extract.

7. Test for Proteins

5ml of plant extract was treated with 10% NaOH solution. After addition of few drops of copper sulphate, formation of reddish violet color confirmed the presence of proteins in extract.

8. Test for Carbohydrate

1ml of extract was treated with 1ml of Benedict's reagent. The mixture was heated on a boiling water bath for 2 minutes solution. Appearance of green color showed the presence of reducing sugar in extract.

9. Test for Anthraquinone

To 200 mg of each extracts, dilute $H_2SO_4$was added and boiled. Then it was filtered and cooled. To the cold filtrate, 3 ml of benzene was added and mixed. The benzene layer was separated and to it, ammonia (2 ml) was added and ammonical layer was observed.

Bacterial Cultures

1. *Escherichia coli* (NCIM-2064)
2. *Pseudomonas aeruginosa* (NCIM-5210)
3. *Staphylococcus aureus* (NCIM-2079)

Agar Well Diffusion Method

Anti-bacterial potential of *Nyctanthes* was tested by Agar well diffusion method. Nutrient agar was autoclaved and poured in the Petri plates under laminar air flow. After solidification of media the bacterial suspension (24 hrs old) was spread over the media. The wells were prepared using cork borer. Extract was dissolved in DMSO (Di Methyl Sulfoxide) in different concentrations such as 25, 50, 100 µg/ml. 40 µl test sample from each concentration was loaded to the wells and incubated for 24 hrs at 37ºC. DMSO was used as a negative control whereas amoxicillin antibiotic disc (10µg) was used as positive control.

Table1: Phytochemicals in the methanolic extract of *Nyctanthes sp.*

S.No.	PHYTOCHEMICALS	INFERENCE
1	ALKALOIDS	PRESENT
2	ANTHRAQUINONES	ABSENT
3	CARBOHYDRATES	PRESENT
4	FLAVONOIDS	PRESENT
5	GLYCOSIDES	PRESENT
6	PHYTOSTEROLS	PRESENT
7	PROTEINS	PRESENT
8	SAPONINS	PRESENT

9	TANNINS	ABSENT
10	TRITERPENOIDS	ABSENT

Table 2: Effect of leaf extract on growth of bacteria *in vitro*.

Bacteria	Methanol extract(µg/ml)			DMSO (Negative control)	Amoxycillin (Positive control)
	25	50	100		
Pseudomonas aeruginosa	-	7	16	-	20
Staphylococcus aureus	-	6	10	-	18
Escherichia coli	-	7	17	-	20

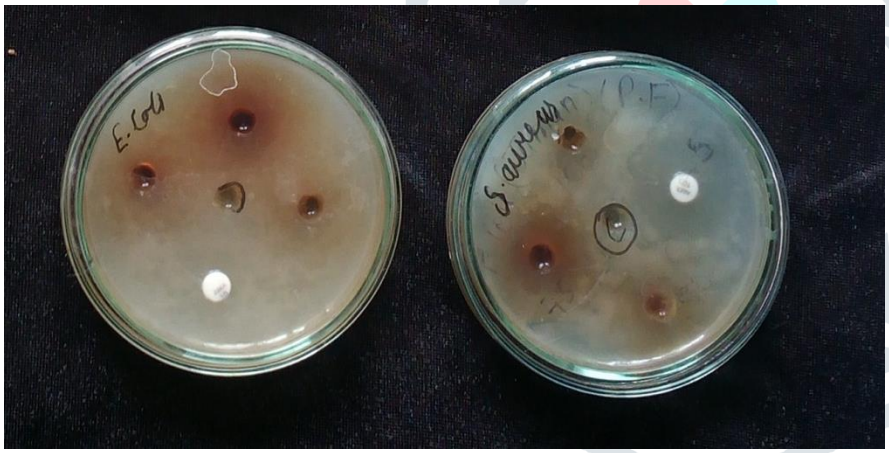

Figure 1: Inhibition zone photographs for mathanolic extract of *Nyctanthes*

RESULTS AND DISCUSSION

The investigation showed (Table 1) that *Nyctanthes arbor-tristis* contains phenolic compounds, glycosides, carbohydrates, proteins, saponins and alkaloids. Triterpenoids, tannins and anthraquinone were absent. In table 2 antimicrobial activity of *Nyctanthes* extract was studied. Results showed that *Escherichia coli* and *Staphylococcus aureus* was highly sensitive to methanolic extract. Antimicrobial activity of extract increases as the concentration increases (Figure-1). The *Nyctanthes* leaf extracts were showing many secondary metabolites are present. Due to these metabolites it also shows antimicrobial activity against many bacteria. The plant contains more metabolites and there is a need for further investigations using fractionated extracts and purified chemical components.

REFERENCES

[1] Singh RB, Jindal VK. Polysaccharide from Nyctanthes arbortristis Linn. Seeds isolation, purification and preliminary analysis of polysaccharide, J Ind Chem Soc. 1985; 62(8):627-28.

[2] Mathuram V, Patra A, Kundu AB. A Phenyl propanoid glycoside from Nyctanthes arbor-tristis, J Ind Chem Soc. 1997; 74:653-55.

[3] Raphael E, 2012. Phytochemical constituents of some leaves extract of Aloe vera and Azadirachta indica plant species. Glo Adv Res J Environ Sci Toxicol 1:14-17.

[4] Kumari SPK, Sridevi V, Lakshmi MVVC, 2012. Studies on Phytochemical screening of aqueous extract collected from fertilizers affected two medicinal plants. J Chem Bio Phy ScIi. 2:1326-1332.

[5] Kubo A Lunde C & Kubo I, 1995. Antimicrobial activity of the olive oil flavor compounds. Jagric Food Chem, 43:1627-33.

[6] Tanaka H, Sato M, Fujiwara S, 2002. Antibacterial activity of isoflavonoids isolated from Erythrina variegate against methicillin-resistant Staphylococcus aureus. Lett Appl Microbial, 35:494-498.

[7] Yadav, S. Gupta, P. and Rastogi, D. 2018. Antibacterial Activity of Aegle Marmelos Leaf Extracts. Int. J. Cre. Res. Tho., 6(2): 879-881.

[8] Yadav, S. 2018. Antibacterial Activity of Garlic. Int. J. Bas. Adv. Res., 4(5): 156-159.