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Anti-Bacterial Activity of *Nyctanthesarbor-tristis* Linn. Leaves

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ABSTRACT

Nyctanthesarbor-tristis Linn. belonging to family Oleaceae is a well-known medicinal plant. The leaves extracts of the plant were tested for their *in vitro* antimicrobial activity by agar well plate method. The test organisms were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Staphylococcus epidermidis*, *Escherichia coli*. The zone of inhibition and Minimum Inhibitory Concentration (MIC) of the extracts were determined and compared with the standard drug of ofloxacin. The Aqueous extract was found to have antibacterial activity. This method is based on the diffusion of an antibiotic from a well through the solidified culture media of a petridish used for study. Growth of inoculated microorganism is inhibited entirely in a circular area "Zone", around the well containing a solution of the antibiotic and the plant extract.

INTRODUCTION

Herbal medicine has become an integral part of standard healthcare, based on a combination of time honoured traditional usage and ongoing scientific research. Burgeoning interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential and safety. *Nyctanthesarbor-tristis* Linn.(NAT) is a small divine ornamental tree used to pray the God according to Indian mythology known across the country for its fragrant white flowers. It is commonly called as nyctanthes means night flowering and arbortristis means as it loses its brightness during day time. It is common wild hardy large shrub or small tree, native to India, distributed wild in sub-Himalayan regions and southwards to Godavari. It is also planted in Indian gardens for ornamental purpose due to its highly fragrant flowers^[1]. It is a shrub or small tree up to 10 m in height with gray to greenish rough bark with stiff whitish hairs.

It has been extensively used as a therapeutic agent in the Ayurvedic healing traditions of South Asia. Traditionally used to treat sciatica, arthritis and malaria, however, reports from the literature have also indicated that the leaf oil from NAT include hepatoprotective, anti-leishmanial, antiviral and antifungal activities. Local people of Andhra Pradesh, India use the whole tree for cancer, root for fever, sciatica, anorexia and bark as expectorant. Other research into the leaf extract of the NAT have shown considerable immunological activity and water soluble ethanol extracts from the leaves are reported to possess anti-inflammatory activity which, however, accompany development of ulcers in test rats. In addition, anti-oxidant studies on the acetone-soluble ethyl acetate leaf extracts have shown significant activity against hydroxy and superoxide radicals, as wells as peroxide scavenging activity^[2].

From ancient civilization various parts of different plants were used to eliminate pain, control Suffering and counteract disease. Most of the drugs used in primitive medicine were obtained from plants and are the earliest and principle natural source

of medicines. The plants used, as drugs are fairly innocuous and relatively free from toxic effects or were so toxic that lethal effects were well known. The nature has provided the storehouse of remedies to cure all ailments of mankind. There is no doubt that plants are a reservoir of potentially useful chemical compounds which serve as drugs, are provided newer leads and clues for modern drug design by synthesis^[3].

MATERIAL AND METHODS

Plant Material

The crude drugs were procured from the local areas of (Bohali) Pandharpur and their identity was confirmed by correlating their morphological and microscopically characters with those given in literature and also by authenticating it from "Botanical Survey of India" Pune.

The collected material was dried in shade. The dried material was stored in air tight poly bag till further use.

Method

Decoctions of *Nyctanthesarbor-tristis* Leaves were done with aqueous solvent. The extracts were dried and evaporated under reduced pressure to give dry powders. The powders were stored in airtight ambered colored glass containers and further used for studies.

Anti-Bacterial Activity

The extracts of *Nyctanthesarbor-tristis* Linn. Leaves of antibacterial activity were evaluated by the agar well plate method. This method is based on the diffusion of an antibiotic from a well through the solidified culture media of a petridish used for study. Growth of inoculated micro-organism is inhibited entirely in a circular area "Zone", around the well containing a solution of the antibiotic and the plant extract.

Minimum Inhibitory Concentration

The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. This was determined from readings on the culture plates after incubation. The most commonly employed methods are the tube dilution method and agar dilution methods. Serial dilutions are made of the products in bacterial and fungal growth media. The test organisms are then added to the dilutions of the products, incubated, and stored for growth. This procedure is a standard assay for antimicrobials.

Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents.

Agar Well Diffusion Method

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 hour old-broth culture of respective bacteria. Wells (5 mm diameter) were made in each of these plates using sterile cork borer. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Test Micro-organisms

The microorganisms used were two each of Gram +ve and Gram -ve human pathogens.

They are:

1. *Staphylococcus aureus* (Gram +ve)
2. *Staphylococcus epidermis* (Gram +ve)
3. *Escherichia coli* (Gram -ve)
4. *Pseudomonas aeroginosa* (Gram -ve)

The organisms were maintained on nutrient agar slants. These were tested using nutrient broth. One loop-full of the respective culture's (*S. aureus*, *Staphylococcus epidermis*, *Escherichia coli* and *P. aeroginosa*) were taken in slants which were maintained below 40 °C were taken and inoculated in the broth and incubated at 37 °C for 24 hrs and were observed for the growth of the organism with naked eye for their turbid nature. It was compared with that of sterile broth. The presence of turbidity indicated growth and suitability of the culture for further work^[4].

Preparation of Stock Culture

From the cultures which are maintained on nutrient agar slants one loopful of the respective organisms were taken and aseptically transferred to 100 ml of sterile nutrient broth in a flask which was shaken thoroughly and incubated at 37 °C for 24 hrs.

Preparation of Test Solution

The test solution of extract was prepared by dissolving 100 mg of dry extracts of the leaves of *Nyctanthesarbor-tristis* Linn in 100 ml of sterile water. Ofloxacin was used as standard in the same concentration as that of the plant extract ^[5,6].

Preparation of Culture Media

The media used for growth of bacteria is, Nutrient agar medium. The media were sterilized by autoclaving at 15 lb/sq. inch pressure at 121 °C for 15 minutes.

Procedure ^[7-13]

Antibacterial activity of different test extracts was screened by agar well plate method

A previously liquefied medium, appropriate for the test is inoculated with the requisite quantity the suspension of the microorganism, the suspension was added to the medium at a temperature between 40-50 °C and the inoculated medium was poured immediately into dried petri-dishes to occupy a depth of 3 to 4 mm.

The dishes were left standing for 1-4 hours, at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. Subsequently incubated for about 18 hrs. At about 37 °C and the diameter of the circular inhibition zones were measured. The data were plotted for graphical representation (**Figures 1 and 2**) of every extract against zone of inhibition and represented in below **Table 1**.

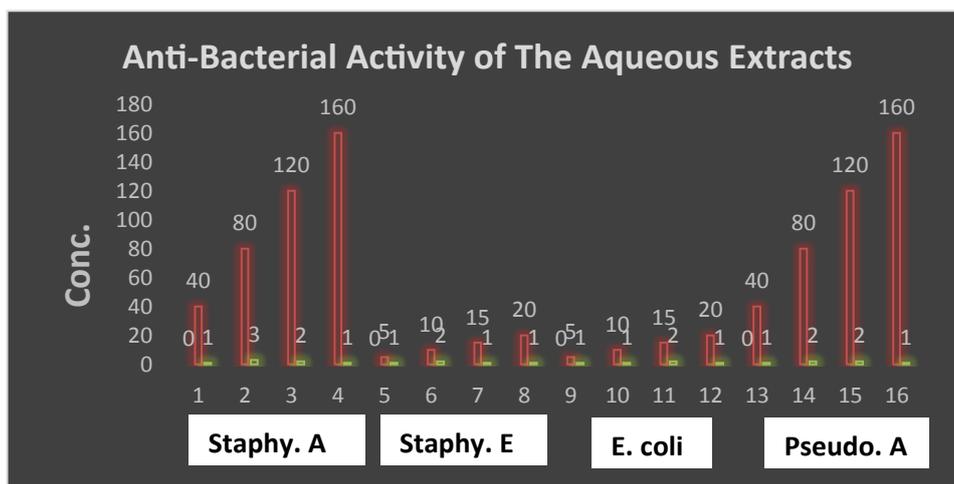


Figure 1. Graph of anti-bacterial activity of the extracts of powdered leaves of *Nyctanthesarbor-tristis* Linn. leaves.

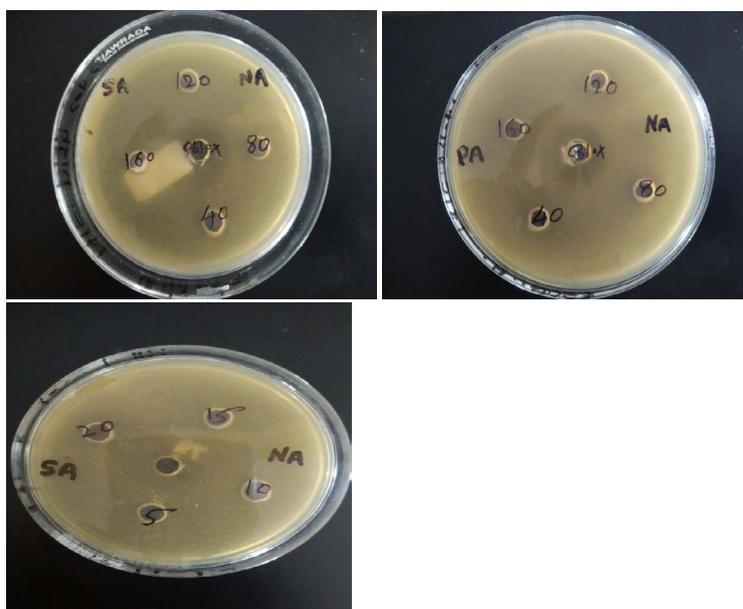


Figure 2. Antibacterial activity of extract of *Nyctanthesarbor-tristis* Linn. leaves.

TEST microorganism	Diameter of zone of inhibition (mm)		
	Standard ofloxacin	Aqueous extract	
<i>Staphylococcus aureus</i> (Gram +ve)	8 mm	Conc.µg/ml	Zone in mm
		40	1 ± 1 mm
		80	3 ± 1 mm
		120	2 ± 1 mm
		160	1 ± 1 mm
<i>Staphylococcus epidermis</i> (Gram +ve)	6 mm	Conc.µg/ml	Zone in mm
		5	1 ± 1 mm
		10	2 ± 1 mm
		15	1 ± 1 mm
		20	1 ± 1 mm
<i>Escherichia coli</i> (Gram -ve)	6 mm	Conc.µg/ml	Zone in mm
		5	1 ± 1 mm
		10	1 ± 1 mm
		15	2 ± 1 mm
		20	1 ± 1 mm
<i>Pseudomonas aeruginosa</i> (Gram -ve)	6 mm	Conc.µg/ml	Zone in mm
		40	1 ± 1 mm
		80	2 ± 1 mm
		120	2 ± 1 mm
		160	1 ± 1 mm

Standard: Ofloxacin 5 µg/ml Dose of test extract: 50 ml.

Table 1. Anti-bacterial activity of the aqueous extracts of powdered leaves of *Nyctanthesarbor-tristis* Linn.

CONCLUSION

Extract of leaves of *Nyctanthesarbor-tristis* Linn were pharmacologically screened for *In vitro* antibacterial activity. It can be concluded that aqueous extract of the leaves of *Nyctanthesarbor-tristis* Linn shows significantly positive *In vitro* antibacterial activity when compared with ofloxacin as standard antibacterial drug. The activity may be due to presence of alkaloids, glycosides, carbohydrates, tannins, gums and mucilage, flavonoids and glycosides in extracts.

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