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Research Article

Antimicrobial Activity of Stem Bark Extracts of *Nyctanthes arbortristis* linn. (Oleaceae)

Vats Manisha*, Sharma Neha and Sardana Satish

Department of Pharmacognosy, Hindu College of Pharmacy, Sonipat-131001, Haryana

Abstract
Nyctanthes arbortristis Linn. belonging to family Oleaceae is a well known medicinal plant.The stem bark extracts of the plant were tested for their *in vitro* antimicrobial activity by cup plate method. The test organisms were *Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger.* The zone of inhibition and Minimum Inhibitory Concentration (MIC) of the extracts were determined and compared with the standard drugs ciprofloxacin and fluconazole. The chloroform extract was found to have both antibacterial and antifungal activity whereas the petroleum ether and ethanol extracts possess only antibacterial activity.

Key words: *Nyctanthes arbortristis,* Oleaceae, Antimicrobial, MIC

Introduction

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries and many infectious microorganisms are resistant to synthetic drugs; hence an alternative therapy is very much needed[1] . Since ages, man has been dependent on nature for curing various body diseases. 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 principal 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 [2]. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [3]. *Nyctanthes arbortristis* Linn. commonly known as Harsinghar or Night Jasmine is one of the well known medicinal plants. Different parts of *N. arbortristis* are known to possess various ailments by rural mainly tribal people of India (Orissa and Bihar) along with its use in Ayurveda, Sidha
and Unani systems of medicines. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic [4, 5, 6]. Leaves are also used in the enlargement of spleen. Traditionally the powdered stem bark is given in rheumatic joint pain, in treatment of malaria and also used as an expectorant [5]. The claimed traditional medicinal uses have been proved on scientific basis using *in-vitro* and *in-vivo* experiments. The plant have been screened for antihistaminic activity, CNS activities (viz. hypnotic, tranquillizing, local anesthetics), analgesic, anti-inflamatory, antipyretic, antiulcer, amoebicidal, anthelmintic, antitrypanosomal to antidepressant, antiviral and immunomodulatory [7]. Leaves extracts was found to have antimicrobial activity [8] but no report is available on the antimicrobial activity on the stem bark part so the present study is aimed at the screening of the antimicrobial activity in the stem bark extracts of the plant *N. arbortristis* Linn.

*Corresponding Author: Vats Manisha
Department of Pharmacognosy, Hindu College of Pharmacy, Sonipat-131001, Haryana
E-mail: ms.vats@yahoo.com

MATERIAL AND METHODS

Plant collection and identification:

The stem bark of *N. arbortristis* Linn. was collected from Sonipat, India in June, 2008. The bark was shade dried at room temperature (30-40 °C). The plant was authenticated at the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, under voucher specimen number NISCAIR/RHMD/Consult/-2008-09/1058/89.

Preparations of Extracts

The plant material was coarsely powdered and extracted sequentially with petroleum ether (60-80°C), chloroform and ethanol (95%) using Soxhlet apparatus. The extracts were filtered and allowed to evaporate to dryness. Each extract was transferred into clean and dried airtight vials until ready for use.

Microorganisms

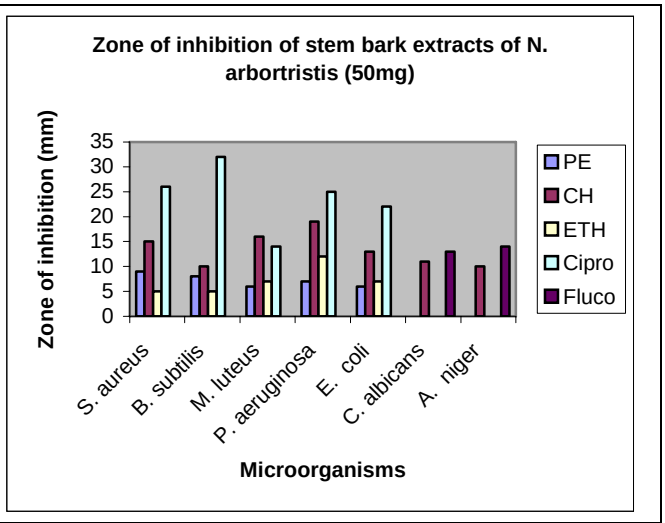
The test organisms were *Staphylococcus aureus* (MTCC 737), *Micrococcus luteus* (MTCC *106), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Candida albicans* (MTCC 3017) and *Aspergillus niger* (MTCC 1344).The microorganisms were availed from M.T.C.C. Institute of Microbial Technology, Sector 39-A, Chandigarh-160036, India. ST No: 02/ST/Sci & Tech/STC/Chd/2002. Invoice No.

Table .1: Antimicrobial activity (zone of inhibition) of stem bark extracts of *N. arbortristis* Linn.

Extracts/ Standards	PE				CH				ETH				Cipro	Fluco
Conc.(mg/ml)	10	20	40	50	10	20	40	50	10	20	40	50	5	5
Microorganisms	Zone of inhibition (mm)													
S. aureus	4	5	8	9	7	9	13	15	1	2	4	5	26	NA
B. subtilis	4	6	7	8	5	6	8	10	-	2	3	5	32	NA
M. luteus	3	5	5	6	6	8	11	16	2	3	6	7	14	NA
P. aeruginosa	4	5	8	7	9	11	14	19	6	8	11	12	25	NA
E. coli	3	4	6	6	8	9	11	13	4	5	6	7	22	NA
C. albicans	-	-	-	-	3	6	8	11	-	-	-	-	NA	13
A. niger	-	-	-	-	4	6	8	10	-	-	-	-	NA	14

PE = Petroreum ether extract, CH = Chloroform extract, ETH = Ethanol extract, Cipro = ciprofloxacin, Fluco = fluconazole, *S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis, M. luteus = Micrococcus luteus, P. aeruginosa= Pseudomonas aeruginosa, E. coli = Escherichia coli, C. albicans = Candida albicans, A.niger = Aspergillus niger,* NA = Not Applicable

MTCC/08/10/5396. The organisms were subcultured onto nutrient agar in order to determine their viability. The identity of each test organism was confirmed using standard cultural, morphological and biochemical techniques [9]. Stock cultures were maintained on nutrient agar slants at 4°C and then subcultures in nutrient broth at 37°C prior to each antimicrobial test



Graph 1: Antimicrobial activity (zone of inhibition) of stem bark extracts of *N. arbortristis* Linn. at a concentration 50mg/ml

Evaluation of antimicrobial activity

I. Culture media Nutrient agar (NA) (Himedia) containing bromocresol purple was used for the activation of *Bacillus* species, while NA was used for other bacteria. Sabouraud glucose agar (Himedia) was used for the activation of the fungi. Nutrient broth was used for MIC determination.

II. Chemicals for antimicrobial assay Ciprofloxacin and Fluconazole (Central Drug House (P). LTD., New Delhi 110002., India) were used as positive reference standards (RA) for all bacterial and fungi strains respectively. The dimethylsulfoxide (DMSO) (Qualigenis) was used as solvent for the tested samples.

III. Assay method

All the experimentation was done in aseptic area under laminar air-flow cabinet. The agar diffusion method [10] was adopted for the study. Broth cultures of the test isolates (0.1 ml) containing 1.0 X 10^5 CFU/ml of organism was introduced into a sterile petri dish and 15 ml of molten nutrient agar were added. The content was thoroughly mixed and then allowed to solidify. The extracts were dissolved in DMSO and used in concentrations 10, 20, 40 and 50 mg/ml. Ciprofloxacin (5µg/ml) was used as standard for antibacterial activity and Fluconazole (5µg/ml) was used for antifungal activity. Holes were bored in the plates, using a standard sterile cork borer of 8 mm diameters and equal volumes of the plant extracts (1000 µl) were transferred into the wells with the aid of micropipette. The experiments were carried out in triplicate. The plates were kept for 1hr for pre-diffusion and incubated at 37°C/24hr (plates containing bacterial cultures), 25°C/3days (plates containing *Candida albicans* culture) and 25°C/7days (for plates containing *Aspergillus niger* culture). At the end of incubation, zone of inhibition was measured in all the plates.

IV. Minimum inhibitory concentration (MIC)

It was determined by tube dilution method (turbimetric method) [11, 12]. 1 ml of the sterilized media was poured in the sterile test tubes. The stock solutions of the extracts having

Table.2: Minimum inhibitory concentration of stem bark extracts of *N. arbortristis* Linn.

Microorganism	PE (µg/mL)	CH (µg/mL)	ETH (µg/mL)	Cipro (µg/mL)	Fluco (µg/mL)
S. aureus	12.5	6.25	12.5	0.625	-
B. subtilis	12.5	6.25	12.5	0.625	-
M. luteus	6.25	6.25	6.25	0.625	-
E. coli	6.25	3.12	6.25	0.312	-
P. aeruginosa	3.12	3.12	3.12	0.312	-
A. niger	12.5	12.5	12.5	-	0.625
C. albicans	12.5	6.26	12.5	-	1.25

concentration of 50 µg/ml were used. The extracts were serially diluted to give a concentration of 25, 12.5, 6.25, 3.12 and 1.56 µg/ml. In all the test tubes 0.1 ml of suspension of bacteria in saline was added and incubated at 37°C/24hr (Plates containing bacterial cultures), 25°C/3days (for plates containing *Candida albicans* culture) and for 25°C /7days (for plates containing *Aspergillus niger* culture). Post-incubation
the plates were observed for turbidity.

RESULTS AND DISCUSSION

Table 1 shows the results of antimicrobial activity against the tested microorganisms. All extracts showed varying degrees of inhibition against all the bacterial stains but only chloroform extract possess antifungal activity against *Candida albicans* and *Aspergillus niger*. Graph 1 shows the comparison of the different extracts at a concentration of 50mg/ml with the standard drugs. Chloroform extract showed higher zone of inhibition as compared with the petroleum ether and ethanol extracts and it is found to be more active against *Pseudomonas aeruginosa* as compared in comparison with the other tested bacteria. The Minimum Inhibitory Concentration (MIC) values of the extracts against tested microorganisms were shown in Table 2. It showed that MIC for *Pseudomonas aeruginosa* is found to be less followed by *Micrococcus luteus* and *Escherichia coli* as compared with other tested microorganisms. Overall chloroform extract of the stem bark of *N. arbortristis* Linn. exhibited significant activity .

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