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ANALYSIS OF THE PHYTOCHEMICAL CONTENT AND ANTIMICROBIAL ACTIVITY OF *NYCTANTHES ARBOR- TRISTIS*

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ABSTRACT

The antimicrobial activity was evaluated on Gram positive bacteria- *Staphylococcus aureus* (MTCC *3160)*, Gram negative-*Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 647) and fungi- *Aspergillus niger* (MTCC282), *Aspergillus flavus* (MTCC 2456)*, Fusarium culmorum* (MTCC349) and *Rhizopus stolonifer* (MTCC 2591). The dried leaf extract prepared in ethanol, methanol, petroleum ether and aqueous solutions were used to assess their antibacterial and antifungal potential in terms of zone of inhibition of bacterial growth. The petroleum ether extract exhibited highest zone of inhibition against *P. aeruginosa* (20.3±0.92 mm) with low MIC value (24.5 mg/ml). Phytochemical screening of the extract showed the presence of alkaloids, carbohydrates, flavonoids, tannins and saponins. Results obtained in the present study indicate that *Nyctanthes arbor-tristis* possess compounds with antimicrobial properties that can be used for plant based antimicrobial agents.

KEY WORDS : ***Nyctanthes arbor-tristis*, antimicrobial activity, zone of inhibition, phytochemical analysis.**

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INTRODUCTION

Plants are known as the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients (Lown, 1993). Plants produce a diverse range of bioactive molecules making them as a rich source of different types of medicines (Stuffness and douros, 1982). Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural product play a vital role in modern drugs development in the pharmaceutical industry (Baker et al., 1995). Antimicrobial activity has been reported in many plants by various workers viz., Sarin, 2005; Bansal et al., 2010; Chahal et al., 2010; Seth and Sarin, 2010 and Malwal and Sarin, 2011. *Nyctanthes arbor-tristis* (family oleaceae) commonly known as night jasmine mainly characterized by the presence of phenylethanoid derivatives and iridoid glycosides (Jensen et al., 2002). It is used in the traditional medicine as stomachic, intestinal astringent, expectorant, piles, various skin diseases and as hair tonic (Khatune et al., 2001). The decoction of leaves is widely used in ayurvedic medicine for the treatment of sciatica and arthritis (Kirtikar and Basu, 1935; Chopra et al., 1958; Nadkarni, 1976). It has also been reported to possess hepatoprotective, anti-leishmanial, anti-viral and anti-fungal activities (Puri et al., 1994) and analgesic, antipyretic and ulcerogenic activities (Saxena et al., 1987). The plant also possess anti-allergic (Gupta et al., 1993), anti-malarial (Badam et al., 1988), antihelminthic (Lal et al., 1976), activities and recently reported hepatoprotective (Hukkeri Kusum et al., 2006), anti-spermatogenic(Gupta et al., 2006) and antioxidant activities (Rathee et al., 2007). The present study was undertaken to investigate the antimicrobial activity and phytochemical analysis of *N. arbor-tristis* in view of its diverse pharmacological application in ancient and modern system of medicine.

MATERIALS AND METHODS

Plant material

Plants of *N. arbor-tristis* were collected from the nursery of University of Rajasthan, Jaipur (RUBL21100). The leaves *N. arbor-tristis* were separated, washed with running water to remove dust, shade dried and powered.

Preparation of extract

The powered leaves (500mg) of *N. arbor-tristis* were extracted with ethanol, methanol, petroleum ether and aqueous solution using soxhlet`s apparatus for 12-14 hours on a water bath separately. The organic extracts were separately filtered with Whatmann No. 1 filter paper and evaporated to dryness on water bath to obtain semi-solid mass. However, aqueous extraction is performed by using hot water maceration. The dried extract was stored at 5ºc in the refrigerator until used for further studies.

Antimicrobial Screening Test microorganisms

Antimicrobial activity was evaluated against common pathogenic microorganisms, Gram positive bacteria- *Staphylococcus aureus* (MTCC *3160),* Gram negative- *Escherichia coli* (MTCC *1652), Pseudomonas aeruginosa* (MTCC *647)* and fungal strains *Fusarium culmorum* (MTCC *349,) Rhizopus stolonifer* (MTCC 2591), *Aspergillus niger* (MTCC *282)* and Aspergillus *flavus* (MTCC 2456). The entire tested microorganism was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. The bacterial cultures were grown and maintained on Nutrient Broth medium at 37ºC for 24h while the fungal culture were maintained on Potato Dextrose Agar slants and incubated at 27ºC for 48h.

Antimicrobial activity

Antimicrobial assay of the crude extracts was performed again nine tested pathogenic strains by disc diffusion method (Gould and Bowie, 1952). The nutrient agar plates and potato dextrose agar plates were seeded with suspension (10^6 cfu/ml) of the bacterial and fungal strains vice-versa. The empty sterilized Whatmann No. 1 filter paper disc (6mm) were impregnated with 1 mg/ml of extracts dried and placed aseptically on seeded plates with the help of a sterile forceps. Finally, the sensitivity discs were pressed with forceps to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 minutes (pre diffusion time). The standard discs (6mm) impregnated with antibiotics gentamycin and nystatin (1.0mg/disc) were used as positive control. The plates were incubated at 37ºC for 24 hr and 25ºC for 48 hr for bacteria and fungi, respectively. The diameter of the inhibition zone (mm) was measured. The experiment was done in triplicate and the mean values (±SD) calculated for conclusion.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method (Basri and Fan, 2005). For broth dilution, 1 ml of standardized suspension of a strain (10^6 cfu/ml) was added to each tube containing extracts at various concentrations in nutrient broth medium. The tubes were incubated at 37ºC for 24h (for bacterial strains) and 25ºC for 48h (for fungal strains) and observed for visible growth after vortexing the tubes gently. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation.

Qualitative Analysis of Phytochemicals

Specific qualitative tests were performed for the presence of phytochemicals viz., alkaloids, carbohydrates, tannins, saponins, flavonoids and triterpenoids in various extracts of *N. arbor-tristis leaves.*

RESULT AND DISCUSSION

The nature has provided a storehouse of natural 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 (Kiew and Baas, 1984). 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 (Iwu et al., 1999). In the present investigation, antimicrobial efficacy of the crude extract of *N. arbor-tristis* was quantitatively assessed on the basis of inhibition zone and minimum inhibitory concentration. The ethanol, methanol, petroleum ether and aqueous extracts of *N. arbor-tristis* exhibited varying degree of inhibitory effect against all tested pathogenic strains (Table 1 and 2). The most susceptible bacterium and fungi are *P. aeruginosa* and *Rhizopus stolonifer*, respectively.The inhibition zones (IZ) were in the range of 8.6±0.52 to 20.3±0.92 mm for most of the tested strains. The MIC of crude extract of leaves was determined at the concentration ranging from 23.9 to 68.3mg/ml. Crude petroleum ether extract of *N. arbor-tristis* leaves showed more pronounced antimicrobial activity as compared to other extracts. The petroleum ether leaves extract exhibited highest zone of inhibition against *P. aeruginosa* and *R. stolonifer* (20.3±0.92 and 19.4±0.65mm, respectively) with low MIC value (24.5mg/ml and 23.9mg/ml, respectively). Presence of important metabolites viz., carbohydrate, tannins, flavonoids, alkaloids, triterpenoids and saponins in leaves extracts were confirmed after performing specific qualitative test (Table3).

Table 1
Antimicrobial activity of leaf of Nyctanthes arbor-tristis (inhibition zone)

Microorganisms	Activity	Extracts in various solvents			
		EtOAc	MeOH	PE	Aqueous
Bacteria					
S. aureus	IZ	12.5±0.65	16.3 ±0.91	18.3 ±0.56	9.4 ±0.55
	AI	0.516	0.673	0.859	0.566
E. coli	IZ	14.6±0.55	19.2 ±0.32	17.8 ±0.71	12.4 ±0.35
	AI	0.603	0.793	0.787	0.656
P. aeruginosa	IZ	15.8±0.45	17.6 ±0.49	20.3 ±0.92	9.6 ±0.69
	AI	0.778	0.866	0.935	0.768
Fungi					
A. niger	IZ	15.2±0.65	18.5 ±0.63	….	11.2 ±0.29
	AI	0.899	1.094	…..	0.805
A. flavus	IZ	10.6±0.71	13.6±0.75	9.5±0.80	13.4±0.51
	AI	0.627	0.804	0.766	0.761
F.oxisporum	IZ	12.4±0.63	14.5±0.59	14.3±0.71	14.5±0.64
	AI	0.716	0.838	0.803	0.671
R.stolonifer	IZ	8.6 ±0.52	13.8±0.49	19.4±0.65	13.8±0.42
	AI	0.497	0.797	0.941	0.901

Abbreviations: IZ= Inhibition zone (in mm) includes the diameter of disc (6 mm); Standards: gentamycin (1.0 mg/disc), nystatin (1.0 mg/disc); AI- activity index = IZ of test sample / IZ of standard. Values are mean of triplicate readings (mean ± S.D).

Table 2
Antimicrobial activity of N. arbor-tristis

Microorganisms		**EtOAc**	**MeOH**	**PE**	**Aqoeous**	**Standard**
Bacteria						
S. aureus	MIC	66.4	50.7	32.1	61.4	23.5
E. coli	MIC	57.7	38.7	39.3	52.4	33.2
P. aeruginosa	MIC	40.2	31.4	24.5	37.1	20.6
Fungi						
A. niger	MIC	28.1	8.6	….	37.5	9.3
A. flavus	MIC	55.3	37.6	41.4	41.9	10
F.oxisporum	MIC	46.4	34.2	37.7	50.9	30.7
R.stolonifer	MIC	68.3	38.3	23.9	27.9	19

TABLE 3
Phytochemical screening of various extract of N. arbor-tristis

Phytoconstituents	Petroleum ether extract	Ethanol extract	Chloroform extract	Ethyl acetate extract
Carbohydrates	--	++	+	+
Tannins	--	+	_	+
Flavonoids	_	_	+++	+
Alkaloids	_	++	+	_
Triterpenoids	+	_	_	_
Saponins	_	++	_	+

CONCLUSION

The present investigation revealed that the various extract from leaves of *N. arbor-tristis* exhibited antimicrobial properties which explain the basis for its use in traditional medicines. However, petroleum ether extract exhibited significant inhibitory activity against tested pathogenic microorganisms.

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