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Antibacterial Activities and Phytochemical Analysis of Different Plant Parts of *Nyctanthes arbor-tristis* (Linn.)*

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Abstract: The antibacterial potential of *Nyctanthes arbor-tristis* was evaluated on gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) bacteria. The dried leaf, flower, fruits and seed extracts prepared in ethyl acetate and chloroform were used to assess their antibacterial potential in terms of zone of inhibition of bacterial growth. Both ethyl acetate and chloroform extract of plant parts have shown the significant antibacterial activity against gram-negative bacteria. Fresh plant materials however, had pronounced antibacterial activity as compared to that of dried plant parts. Synergetic effect of combined extract (leaf, flowers, fruit and seeds) on bacteria was less effective as compared to those of individual extract. Furthermore, the antibacterial potential of the extracts were found to be dose dependent. These activities of plant parts were due to the presence of various plant secondary metabolites viz., glycosides and phenolics. The glycosidic and phenolic content measured spectrophotometrically fluctuates markedly among different plant parts.

Key words: *Nyctanthes arbor-tristis*, zone of inhibition, antibacterial activity, glycosides, phenolics

INTRODUCTION

Nyctanthes arbor-tristis (family: Oleaceae) commonly known as Night jasmine or coral jasmine, mainly characterized by the presence of phenylethanoid derivatives and iridoid glycosides (Jensen *et al.*, 2002). It is used in traditional medicine as stomachic, carminative, intestinal astringent, expectorant, in biliousness, 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; Shri Gulabkumaverba Ayurvedic Society, 1949; 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). Roots are used for emaciation and stem bark of this plant is taken to cure dysentery, ulcer of palate and internal injuries (Gupta *et al.*, 2006). Saxena *et al.* (2002) have reported the tranquilizing, antihistaminic and purgative activity of *Nyctanthes arbor-tristis* leaf extract.

The *N. arbor-tristis* demonstrate diverse pharmacological and biological activities like anti-inflammatory (Saxena *et al.*, 1984), analgesic, anti-pyretic along with ulcerogenic (Saxena *et al.*, 1987) activities. The plant also possess anti-allergic (Gupta *et al.*, 1993), anti-malarial (Badam *et al.*, 1988; Misra *et al.*, 1991), leishmanicidal (Singh *et al.*, 1992; Tandon *et al.*, 1991), amoebicidal (Chitravanshi *et al.*, 1992), 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).

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The present study was undertaken to investigate the antibacterial activity of *N. arbor-tristis* in view of its diverse pharmacological application in ancient and modern system of medicine. Flowers, leaves, seeds and fruits ethyl acetate and chloroform extracts investigated for the antibacterial screening. The seed and fruit extracts, however investigated for the first time.

MATERIALS AND METHODS

Plant

Nyctanthes arbor-tristis fresh flowers, leaves, fruits and seeds were collected from Arcot in Vellore district of Tamil Nadu during the month of December (2006) to February (2007). The materials were immediately brought to School of Biotechnology, Chemical and Biomedical engineering, VIT University, Vellore-632 014, India, where the experiments conducted.

Preparation of the Extract

Fresh and dried flowers, leaves, fruits and seeds were collected, weighed (4 g each), washed and macerated in 10 mL of ethyl acetate and chloroform separately. The mixtures were kept for 6 h at room temperature. The mixtures were then filtered through sterile Whatmann filter paper No. 1. Filtrate, thus obtained were centrifuged at 5000 rpm for 5 min. The supernatants were collected in the beaker and the solvents were evaporated to dryness, the residue left over was stored at 4°C in refrigerator. At the time of antibacterial assays the residue was dissolved in 1-3 mL of DMSO.

Preparation of Microorganisms

The organisms used in this study were *Escheichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Klebsiella pneumoniae* (ATCC 2719) and *Staphylococcus aureus* (ATCC 25923). The strains were maintained on nutrient agar slants at 4°C. A loopful of each bacterial strain was inoculated into 50 mL of sterile nutrient broth in 100 mL conical flask. The flasks were incubated on a rotary shaker for 24 h to activate the strain. Mueller Hinton Agar medium was used as bacterial culture medium in the antibacterial assays.

Antibacterial Activity

The antibacterial activity of *Nyctanthes arbor-tristis* ethyl acetate and chloroform extracts were evaluated by agar well diffusion method (Bauer *et al.*, 1966). Twenty four hours broth cultures of the bacteria used for the assay. A sterile cotton swab was dipped into the bacterial suspension and evenly streaked over the entire surface of sterile Mueller Hinton agar plate to obtain uniform inoculum. Wells were punched on the seeded plates using sterile borer (8 mm). The plates were allowed to dry for 5 min. Ethyl acetate and chloroform extracts (100, 200 and 300 µL) were dispensed into each well using sterile micropipette. Streptomycin (10 µL) was used as positive control. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of zone of inhibition (mm).

Extraction and Analysis of Glycosides and Phenolics

The glycosides were extracted according to Ganjewala *et al.* (2000). The dried leaves, fruits and seeds powder (1 g each) first extracted three times with warm hexane (20 mL) for removal of pigments. The hexane extract was centrifuged at 5000 rpm for 5 min and the pellets were extracted twice with warm methanol (20 mL) and centrifuged at 5000 rpm for 5 min after each methanol treatment. The supernatant was collected in a conical flask and methanol was evaporated to dryness. The residues contained glycosides were analyzed by thin layer chromatographic procedure. Thin layer chromatography was performed using pre-coated silica gel 60 F₂₅₄ TLC plate. The mobile phase

contained ethyl acetate: methanol: water (60:14:10, v/v/v). The spots were visualized after spraying the developed TLC plate with vanillin sulfuric acid reagent and incubating the plates at 110°C for 5 min.

Phenolic content of the dried leaves, fruits and seeds (1 g each) were extracted using 10 mL of 0.3 M methanolic HCl. The supernatant obtained after centrifuging the mixture at 5000 rpm for 5 min was collected and evaporated to dryness. The residues were dissolved in 5 mL of distilled water. Aliquots (0.1 mL) of the extracts were transferred to the test tubes and made up to 7.0 mL with distilled water and stirred well. The Folin-phenol reagent (0.5 mL) was added to the solution in test tubes and shaken vigorously. After 3 min, 1 mL of 35% sodium carbonate solution was added. The mixtures were shaken and allowed to stand for 1 h. The absorbance was recorded at 630 nm.

Qualitative Analysis of Phytochemicals

Specific qualitative tests were performed for the presence or absence of phyto-chemicals viz., alkaloids, saponins, phytosterols, tannins, phlobatannins, flavonoids, terpenoids and cardiac glycosides in leaf, fruit and seed extract.

RESULTS

Antibacterial Activities of Different Plant Parts

Antibacterial potential of flower, leaf, seed and fruit ethyl acetate and chloroform extract was assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Table 1 and 2. 100, 200 and 300 µL of each extract was used for antimicrobial screening. The antibacterial activity of extract increased linearly with increase in volume of extract (µL). Both, ethyl acetate and chloroform extract (volume 300 µL) have shown significant antibacterial activity against the microorganisms tested. The results revealed that the gram negative (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria were more sensitive as compared to those of gram positive bacteria to all the extracts. The growth inhibition zone measured ranged from

Table 1: Antibacterial activities of *Nyctanthes arbor-tristis* fresh flower, leaves, seeds and fruits extracts

Bacterial strain	Zone of inhibition of growth (mm)										Streptomycin (10 µg disc ⁻¹)
	Ethyl acetate extract (300 µL)					Chloroform extract (300 µL)					
	Flower	Leaves	Seeds	Fruits	Combined	Flower	Leaves	Seeds	Fruits	Combined	
Gram negative											
<i>E. coli</i>	25	20	R	R	19	22	17	R	14	20	16
<i>P. aeruginosa</i>	19	21	15	20	19	R	18	26	20	21	---
<i>K. pneumoniae</i>	22	20	16	17	21	26	14	16	14	17	18
Gram positive											
<i>S. aureus</i>	14	R	R	R	R	R	R	15	R	13	22

R = Resistant

Table 2: Antibacterial activities of *Nyctanthes arbor-tristis* dried leaves, seeds and fruits extracts

Bacterial strain	Zone of inhibition of growth (mm)									Streptomycin (10 µg disc ⁻¹)
	Ethyl acetate extract (300 µL)				Chloroform extract (300 µL)					
	Leaves	Seeds	Fruits	Combined	Leaves	Seeds	Fruits	Combined		
Gram negative										
<i>E. coli</i>	19	17	21	19	15	20	27	20	16	
<i>P. aeruginosa</i>	R	24	18	19	18	24	20	22	---	
<i>K. pneumoniae</i>	17	15	19	21	13	17	16	19	18	
Gram positive										
<i>S. aureus</i>	R	13	R	R	11	R	R	11	R	

R = Resistant

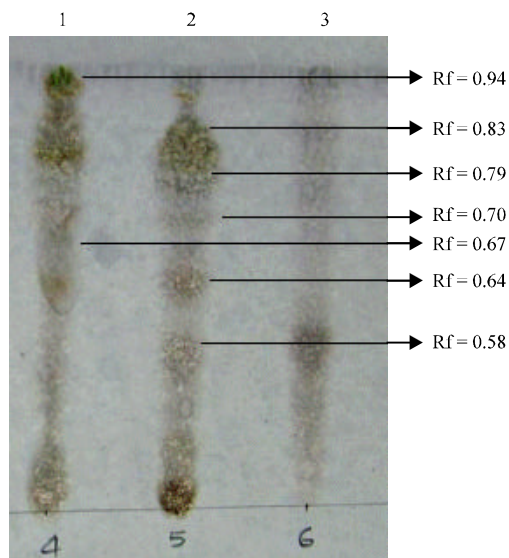


Fig. 1: *Nyctanthes arbor-tristis* glycosidic profile in thin layer chromatographic plate. (1) leaves (2) seeds and (3) fruits

15 to 25 mm for all the sensitive bacteria. Flower ethyl acetate and seed chloroform extract have shown significant broad spectrum antibacterial activity against gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) as well gram-positive (*Staphylococcus aureus*) bacteria. Leaf extract showed antibacterial activity against all the gram negative bacteria tested. Fruit and seed ethyl acetate and flower and seeds chloroform extracts have shown the inhibitory effect against only two of the gram negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). *E. coli* was resistant to fresh fruit and seed ethyl acetate extract. *E. coli* and *Pseudomonas*, however, were most sensitive to the fresh fruit and seed chloroform extracts, respectively. In general, it was observed that fresh plant parts had more antibacterial activity as compared to that of the dried plant parts. Dried leaves, fruits and seeds chloroform extract only had strongest antibacterial activity against gram negative *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, while that of dried leaves and seed extract in ethyl acetate had broad spectrum antibacterial activity.

Antibacterial activity of combined extracts containing equal amount of leaf, fruit and seed ethyl acetate and chloroform extracts have also been evaluated. The combined extract showed less antibacterial activities as compared to antibacterial activity of extract in individual. Similar to individual extracts, combined ethyl acetate extract had more antibacterial activity against gram negative bacteria. However, combined chloroform extract has shown less effect against gram positive *S. aureus*.

Phytochemical Analysis

Presence of some important secondary metabolite viz., alkaloids, phenolics, flavanoids, glycosides, saponins and tannins in leaves, fruits and seeds extract were confirmed after performing specific qualitative tests (Table 3). Alkaloids, however not present in leaf extract. Moreover, Phenolic content was measured spectrophotometrically and glycosides were analyzed by thin layer chromatographic procedure. Phenolic content (mg g^{-1} dry weight) was found to be maximum in seeds and minimum in fruits. Seed's phenolic content (mg g^{-1} dry weight) was almost double than leaf while four fold higher to that of fruit's phenolic content. Thin layer chromatographic analysis have shown the similarity between glycosidic profile of leaf and seed while that of fruit did not match with either of leaf and seed glycoside profiles (Fig. 1).

Table 3: Phytochemical composition of *Nyctanthes arbor-tristis* leaves, seeds and fruits

Plant parts	Phytochemicals								
	Alkaloid	Phyto-sterols	Phenolics	Tanins	Phlobatannins	Flavonoids	Steroids	Glycosides	Saponins
Leaves	-	+	+	+	-	+	+	+	+
Seeds	+	+	+	+	-	+	+	+	+
Fruits	+	+	+	+	-	+	-	+	+

+ = Present; - = Absent

DISCUSSION

The interest in medicinal and aromatic plants has been shown all over the world because of safe and effective constituents of plant products and in particularly the presence of active principles of medicinal plants. In the present study antibacterial properties of *N. arbor-tristis* fresh and dried flowers, leaves, fruits and seeds have been investigated. Both of ethyl acetate and chloroform extracts possess significant antibacterial activity against gram-negative and gram-positive bacteria. The results presented are consistent to those of earlier reported in *N. arbor-tristis* (Khatune *et al.*, 2001, 2005). These results, however did not match with those described by Nair *et al.* (2005). Nair *et al.* (2005) used whole plant water and methanol extracts which was less effective against bacteria tested. The antibacterial activity of *N. arbor-tristis* seed and fruits (fresh and dried) ethyl acetate and chloroform extract reported here is for the first time hence in addition to those reported earlier. The seed and fruits have significant antibacterial activity against the microorganisms tested.

The results of the present studies indicate that the fresh plant material extracts is more effective as compared to those of dried plant extracts. The differences in the antibacterial effects of plant materials used are expected due to the differences in their phyto-chemical compositions. Moreover, the fresh plant part's extracts are more effective as compared to the dried ones. The drying may have caused conformational changes to occur in some of the chemical constituents present in these plant parts (Nair *et al.*, 2005).

Also, the results indicate that both ethyl acetate and chloroform extracts are more effective against gram negative as compared to those of gram positive bacteria. The reason for the different sensitivity between gram-positive and gram-negative bacteria may be attributed to the morphological differences between these microorganisms. Cell wall of gram-negative bacteria is consisted of phospholipids and lipopolysaccharide, hence impermeable to lipophilic solutes (Nikaido and Vaara, 1985). In spite of this permeability barrier the ethyl acetate and chloroform extract exert strong inhibition on gram-negative bacteria. Gram-positive bacteria should be more susceptible because of only an outer layer of peptidoglycan, however, they are resistant against both ethyl acetate and chloroform extract. Only flower, leaf and seed extracts exerts some inhibition on gram-positive bacteria and have broader spectrum of inhibitory activity than the other (fruit) extract.

Phytochemical analysis of leaf, fruit and seeds of *N. arbor-tristis* revealed the presence of phytosterols, phenolics, tannins, flavonoids, glycosides and saponins. The secondary metabolites present in *N. arbor-tristis* are known to be biologically active. Tannins have been found to form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. This activity was exhibited against test organisms with all the plant extracts. Tannins have important roles such as stable and potent antioxidant (Trease *et al.*, 1983), astringent and for treating diarrhea and dysentery (Dhamananda, 2003). The alkaloids the largest groups of chemicals produced by plants have many biological activities. Therefore the antibacterial activities of extracts are expected. Flavonoids are phenolic structures containing one carbonyl group complexes with extra-cellular and soluble protein and with bacterial cell wall. Thus, exhibits antibacterial activity (Cowan, 1999).

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