Phytochemical, Antioxidant and Antimicrobial study of Flowers of *Nyctanthes arbor-tristis* Linn.

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Summary

Nyctanthes arbor-tristis flowers were collected in the month of August from the Manipal region of the India. The flowers were shed dried, powdered and extracted with ethanol in soxhlet apparatus. Dried ethanolic extract was further subjected to fractionation by using petroleum ether, diethyl ether and ethyl acetate solvents in the order of increasing polarity. Phytochemical screening of flower extract showed the presence of volatile oils, phenolic compounds and iridoids. Petroleum ether and diethyl ether fraction showed the significant antioxidant activity. Among the fractions tested for antibacterial activity, petroleum ether and diethyl ether showed moderate antibacterial activity against both gram positive and gram negative species. None of the tested fraction showed significant antifungal activity.

Key words: *Nyctanthes arbor-tristis* linn., phytochemical screening, antioxidant, antibacterial, antifungal

Introduction

Nyctanthes arbor-tristis Linn, family Oleaceae, also known as night jasmine or coral jasmine or paarijaata is a large shrub or a small tree, upto 10 m in height, found in sub-Himalayan region, Madhya Pradesh and southwards of Godavari River in India. The leaves, flowers, seeds and bark of the plant have been reported to possess medicinal principles. The oil from the *N. arbor-tristis* flowers contains α-pinene, p-cymene, 1-hexanol, methylheptanone, phenylacetaldehyde, 1-decanol and anisaldehyde[1]. The acetone extract of the corolla tubes have shown the presence of β-monogentiobioside ester of α-crocetin as a major component and β-digentiobioside ester of α-crocetin as a minor component[2]. *N. arbor-tristis* have been reported for various activities, e.g. alcoholic extract from the leaves for anti-inflammatory[3], antitrypanosomal[4], chloroform and ethylacetate extract from flowers for antibacterial[5], and ethanolic extract from the flowers for antimalarial activity[6]. In the present study, *N. arbor-tristis* flowers were shed dried, powered and subjected to soxhlet extraction in ethanol for 72 h. Ethanolic extract was further fractionated as petroleum ether, diethyl ether, and ethyl acetate fraction. Phytochemical screening of ethanolic extract of the flowers indicates the presence of volatile oil, phenolic principles and iridoids while, fats, glycosides, flavonoids, and alkaloids were found to be absent in ethanolic extract of the flowers. All three fraction obtained were studied for antioxidant and antimicrobial studies.

Materials and Methods

Collection of *N. arbor-tristis* flowers

The flowers of the plant were collected in the month of August from Manipal region of the India and were shed dried.

Preparation of ethanolic extract

The dried flowers were powdered and were subjected to soxhlet extraction in ethanol for 72 h. After extraction, solvent was recovered by distillation and residue remaining behind was collected and dried in desiccator. Yield of ethanolic extract was found to be 20% w/w.

Fractionation of ethanolic extract

50 g of ethanolic extract was suspended in 200 mL of water, followed by successive extraction with solvents in increasing order of polarity i.e. petroleum ether, diethyl ether, ethyl acetate. Organic layers were washed with distilled water and dried over anhydrous sodium sulphate. Finally solvents were removed by distillation and fractions obtained were subjected to drying in desiccator.

Phytochemical screening of ethanolic extract[7]

Ethanolic extract from the flowers of *N. arbor-tristis* was screened for the presence of fats, oils, phenols, glycosides, flavonoids, alkaloids and iridoids by different tests mentioned in literature.

In vitro antioxidant studies

1,1-Diphenyl-2-picrylhydrazyl radical scavenging assay[8]

0.5 mL of test fraction or standard solution (2, 4, 8, 16, 32, 64, 128, 256, 512 and 1000 µg/mL) was added to 0.5 mL of DPPH solution in test tubes. Control test tubes were loaded with 0.5 mL of methanol and 0.5 mL DPPH. The tubes were incubated at 37^{o}C for 30 mts without exposing to light and the absorbance of each solution was measured at 517 nm in Ultra-Violet spectroscopy. Experiment was performed in triplicate. Ascorbic acid was used as a standard. The percentage

scavenging by test fractions at each concentration was calculated by using the formula: (control – test/control) × 100.

Nitric oxide scavenging assay[9]

Griess reagent was prepared by mixing of solution-1 (1% sulphanilamide in 25% v/v hydrochloric acid) and solution-2 (0.01% napthyl ethylenediamine) in equal volumes. Sodium nitroprusside (5mM) in standard phosphate buffer (0.025 M, pH-7.4) solution was incubated with different concentrations (2-1000 µg/mL) of fractions at 37˚C for 5 h. Control was performed in identical manner but without the test fraction. After 5 h, 0.05 mL of incubated solution was removed and diluted with 0.5 mL of Griess reagent. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylenediamine was read at 546 nm. The experiment was repeated in triplicate. Ascorbic acid was used as a standard. The percentage scavenging was calculated as: (control – test/control) × 100.

ABTS scavenging assay[10]

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12 h before use. For the study, different concentrations (2-1000 µg/mL) of the test fractions (0.5mL) were added to 0.3 mL of ABTS solution and the final volume was made with methanol to 1 mL. Ascorbic acid was used as a standard. The absorbance was read at 745 nm and the percentage inhibition was calculated by using the formula, (control – test/control) × 100.

Superoxide scavenging activity[11]

A mixture containing 60 mM, 0.2 mL EDTA (4.47 mg in 10 mL water), 53 µM, 0.25 mL Riboflavin (31.92 mg in 100 mL distilled water), 10 mM, 0.25 mL hydroxylamine HCl (0.114 gm in 100 mL distilled water) and 2 mL phosphate buffer pH 7.4 was added to different concentrations (1.3 mL each) of the test fractions. The above solutions were incubated for 30 m at room temperature. Then 1 mL Griess reagent was added to all the test tubes. Absorbance was measured after 20 mts, at 540 nm. Ascorbic acid was used as a standard. The experiment was performed in triplicate. The percentage inhibition was calculated by using formula: (control – test/control) × 100.

Reduction of ferric ions by ortho-phenanthroline colour method[12]

The reaction mixture containing 0.05%, 1 mL o-phenanthroline in methanol, 200 µM, 2 mL ferric chloride, and various concentrations (2 mL each) of the test fractions were incubated for 10 mts and the absorbance of the same was measured at 510 nm. The experiment was performed in triplicate. Ascorbic acid was used as a standard. The percentage inhibition was calculated by using formula, (control – test/ control) × 100.

Antibacterial activity[13]

The test fractions were screened for their antibacterial activity at 30 µg/mL against two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) species using antibiotic assay medium no.-1, seed agar by Kirby-Bauer disc diffusion method. Ciprofloxacin 30 µg/mL was used as standard.

Antifungal activity[14]

The test fractions were screened for their antifungal activity at 30 µg/mL against *Candida albicans* and *Aspergillus niger* using sabouraud dextrose agar medium by Kirby-Bauer disc diffusion method. Nystatin 30 µg/mL was used as standard.

Results and discussion

Phytochemical screening

Different tests were performed to screen the chemical constituents present in the flower of *N. arbor-tristis*. Test ethanolic extract showed the presence of volatile oils, phenolic compounds and iridoid principles in the flower.

Antioxidant activity

Antioxidant activity of all the three fractions was performed at different concentrations ranging from 2-1000 µg/mL. Ascorbic acid was used as standard in all five models. Among the fractions tested, petroleum ether fraction was found to be most active, showing IC_{50} <100 µg/mL in all the methods except o-phenanthroline scavenging activity model where, IC_{50} was found to be above 1000 µg/mL. Petroleum ether fraction showed IC_{50} of 62.87, 95.47, 62.80, and 86.63 µg/mL in DPPH, nitric oxide, ABTS, superoxide dismutase scavenging activity models respectively. Diethyl ether fraction also showed the promising antioxidant activity with highest IC_{50} being the 52.48 µg/mL, exhibited by ABTS model. In all the five models for antioxidant activity performed, diethyl ether fraction showed IC_{50} less than 350 µg/mL. None of the model for ethyl acetate fraction showed IC_{50} less than 200 µg/mL.

Table-1: Antioxidant activity of petroleum ether, diethyl ether and ethyl acetate fractions

S. No.	Fraction	IC_{50} (µg/mL)				
		DPPH radical scavenging activity	Nitric oxide scavenging activity	ABTS scavenging activity	Superoxide dismutase scavenging activity	o-Phenanthro-line scavenging activity
1	Petroleum ether	62.87	95.47	62.80	86.63	>1000
2	Diethyl ether	89.05	146.95	52.48	131.23	312.27
3	Ethyl acetate	249.19	258.10	>1000	254.52	>1000
4	Ascorbic acid	12.45	16.93	15.04	22.19	18.93

Antibacterial activity

Antibacterial activity of all the three fractions was performed against two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) species. The diethylether fraction showed highest antibacterial activity while petroleum ether fraction was found to be moderately active. Ethyl acetate fraction showed no activity at all against all the four antibacterial species used.

Table-2: Antibacterial activity of petroleum ether, diethyl ether and ethyl acetate fractions

S. No.	Fraction	Zone of inhibition (mM)			
		Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
1	Petroleum ether	10	11	12	14
2	Diethyl ether	15	12	13	15
3	Ethyl acetate	-	-	-	-
4	Ciprofloxacin	28	31	30	28

Antifungal activity

All the three, petroleum ether, diethyl ether and ethyl acetate fractions were screened for antifungal activity against *Candida albicans* and *Aspergillus niger* species. Diethyl ether and ethyl acetate fraction showed moderate activity against *C. albicans* but failed to show against *A. niger*. Ethyl acetate fraction was found to be inactive against both of the fungal species used.

Table-3: Antifungal activity of petroleum ether, diethyl ether and ethyl acetate fractions

S. No.	Fraction	Zone of inhibition (mM)	
		Candida albicans	*Aspergillus niger*
1	Petroleum ether	10	-
2	Diethyl ether	11	-
3	Ethyl acetate	-	-
4	Nystatin	24	20

Conclusions

Among the fractions tested, petroleum ether and diethyl ether fractions showed the promising antioxidant activity. Presence of phenolic compounds in petroleum ether and diethyl ether fractions might have exhibited the antioxidant property. We suggest that, antioxidant activity of *Nyctanthes arbor-tristis* flower should be studied further on the *in vitro* and *in vivo* models. Moderate antibacterial activity was showed by petroleum ether and diethyl ether fractions but no significant antifungal activity was exhibited by any of the fraction tested. Phytochemical screening have showed the presence of volatile oils in the ethanolic extract, could be assigned for antibacterial activity of the flower.

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