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Evaluation of antioxidant and Anthelmintic activity of methanolic flower extract of Nyctanthes arbor-tristis

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ABSTRACT

Nyctanthes arbour-tristis is a shrub belongs to the family Oleaceae. The flowers of this plant are fragrant since the presence of flavonol glycosides. It has also been reported for the presence of β-sitosterol, iridoid glycosides, tannins etc., and known to have immunostimulant, hepatoprotective, anti-viral and antifungal activities. In the present study an attempt is made to identify antioxidant capacity and anthelminthic potential of methanolic flower extract of Nyctanthes arbour-tristis. Antioxidant activity was evaluated by total phenolic content assay, total flavonoids content assay, free radical scavenging activity and reducing ability methods. Anthelmintic activity was evaluated on Perithima posthuma using Piperazine citrate as standard drug. The results obtained for the above activities reveals that Nyctanthes arbour-tristis shows considerable antioxidant activity for all the methods and anthelminthic potential at 300 mg/ml.

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INTRODUCTION

The chief constituents of Nyctanthes arbour-tristis are flavonoid glycosides and iridoid glycosides (Stuppner H, 1993). Flavonol glycosides are known antioxidants (Subash kalija, 2012). They help to remove free radicals formed during metabolic and biological oxidation processes. Antioxidants play a vital role in immunity development and enhance resistance against many bacterial and viral infections. Antioxidant herbs are well verse for their cytotoxic potential against several tumerogenic and cancer cell lines, whereas iridoid glycosides are monoterpene glycosides and also possess bitter principles. These bitter glycosides generally enhance the secretions of gastric juices and anthelminthic activity as they produce excessive and chronic stimulation of cell membrane of helminths that leads to paralysis. In the present research, the author has measured the antioxidant potential of methanolic flower extract of Nyctanthes arbour-tristis by assay methods such as total phenolic content assay, total flavonoids content assay Free Radical Scavenging Assay (FRSA), Reducing ability methods.

Anthelmintic activity was evaluated on Perithima posthuma using methanolic extract of Nyctanthes arbour-tristis at various concentrations and so compared with Piperazine citrate as standard drug. The methods and results were described in the manuscript elsewhere.

MATERIALS AND METHODS

Materials

Collection of plant material

The whole stem part of Nyctanthes arbour-tristis was collected from a rocky terrain near Nambulapulakunta village, Kadiri (Town), Anantapuramu district, Andhra Pradesh, India. Voucher specimens were deposited in S.K. University Herbarium (SKU) (Acc. No. SKU 51293).

Chemicals

Folin-ciocalteu's reagent, Gallic acid, Ascorbic acid were purchased from Sigma-Aldrich (Bengaluru, India).

Methods

Preparation of extracts

The dried and powdered whole stem part of Nyctanthes arbour-tristis was passed through a sieve no.22 and each kilogram of powder was extracted successively by cold percolation (Sukhdev Swami Handa, 2010) with 2.5 litres of methanol. By using rotary vacuum evaporator the extracts were concentrated to dryness under reduced pressure and used for further investigations.

Chemical tests for flavonoids
Shinoda Test (Magnesium Hydrochloride reduction test)

The extract solutions were added with few pieces of magnesium ribbon and HCl. After certain period of time Pink scarlet, colour appeared.

Zinc-Hydrochloride reduction test

The extract solutions were added with a combination of zinc dust and concentrated HCl Red colour was observed after few minutes.

Alkaline reagent test

Addition of few drops of sodium hydroxide solution to the extract solutions, resulted in intense yellow colour formation, upon addition of few drops of dilute acetic acid discoloration was observed.

Antioxidant activity

Determination of total phenolic content by Folin-Ciocalteu's method

The concentration of phenolics present in the methanolic extract was determined using Folin-Ciocalteu's reagent spectrophotometrically. 1mg/ml methanolic extract solution was used in the analysis. To the 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-ciocalteu's reagent which was dissolved in water and 2.5 ml of 7.5% sodium bicarbonate solution were added. Blank solution was also prepared in the same manner by omitting the addition of plant extract. At 45°C the extract samples were thereafter incubated in a thermostat for 45 min. By using UV spectrophotometer at 710 nm the absorbance was determined. Each analysis was performed in triplicate and the mean value of absorbance was obtained. The standard Gallic acid solution was prepared by the same procedure (Standard curve y = 7.012x – 0.0181, r2 = 0.999). Gallic acid equivalent (mg of GAE/g of extract) was used to express the content of phenolics in the extract. The values obtained for the concentration of total phenols are expressed as mg of GAE/g of extract. (Maryam Mohammadi, 2016).

Determination of flavonoid content by UV spectrophotometric method

The content of flavonoids present in methanolic extract was determined spectrophotometrically. 1mg/ml methanolic extract solution was used in the analysis. To 1 ml of 1mg/ml sample solution 0.5 ml of 2% ethanolic aluminum chloride solution was added and the solution was kept at room temperature for 1 hr. Blank solution was also prepared in the same manner by omitting the addition of plant extract. By using UV spectrophotometer the absorbance was measured at 420 nm. Each analysis was performed in triplicate and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin (Standard curve y = 16.213x – 0.0581, r2 = 0.999). The content of flavonoids in the extract was expressed in terms of rutin equivalent (mg of RUE/g of extract). (Maryam Mohammadi, 2016).

Reductive Ability

Oyaizu method was used for determining the Reductive ability (I.F. Benzie, 1996) in methanolic extract of Nyctanthes arbour-tristis by dissolving in 1ml of distilled water, to this 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of Potassium ferric cyanide [K3Fe(CN)6] (1%) were added followed by centrifugation at 3000 rpm for 10 min. 2.5 ml of distilled water and 0.5ml of FeCl3 (0.1%) was added to the 2.5 ml of the upper layer of the solution, measurement of absorbance at 550nm. Reference compound as Butylated Hydroxyl Toluene (BHT). Analysis was performed in triplicate. Re-

$$= \frac{(V0-V1)}{V0} \times 100$$

V_0= absorbance of control and the V_1= absorbance of the sample.

ducing ability (%) was calculated according to formula (1).

Formula 1

Free Radical Scavenging Activity (FRSA) using hydrogen peroxide

Czochra and Widwnsk method was used for performing FRSA (H. Meena, 2014) for the methanolic extract. According to this method 2 ml of hydrogen peroxide (30 %) and 2.4 ml of 0.1 M phosphate buffer (pH 7.4) were added to 1.0 ml of methanolic sample (100 µg / ml), kept aside for 10 min followed by recording of absorbance at 230 nm. Analysis was carried out in triplicate. Blank

Table 1: Phytochemical analysis of methanolic extract of *Nyctanthes arbour-tristis*

Phytoconstituents	Methanolic extract of *Nyctanthes arbour-tristis*
Phenolics	+ ve
Flavonoids	+ ve
Tannins	+ ve
Terpenes	+ ve
Alkaloids	+ ve
Glycosides	+ ve
Vitamins (A & D)	+ ve
Calcium, Magnesium, Zinc	+ ve

Table 2: Results of *In vitro* antioxidant activity tests

Name of the Assay	Content present in methanolic extract *
Total phenolic content	34.5 ± 0.03 mg GAE/g
Total flavonoid content	20.2 ± 0.17 mg RUE/g.
FRSA	90.32 % ± 0.37
Reductive ability	98.19 % ± 0.24

Table 3: Anthelminthic activity

Groups	Concentration (mg/ml)	Earthworms Paralysis time	Earthworms Death time
Control (Distilled water)	--	--	--
Nyctanthes arbour-tristis extract	100 mg/ml	3 min 30 sec	9 min 30 sec
	200 mg/ml	3 min 30 sec	8 min
	300 mg/ml	3 min 30 sec	7 min
Standard (Piperazine citrate)	10 mg/ml	19 min	52 min

was prepared without adding hydrogen peroxide and control was prepared without a sample. Standard compound used was Ascorbic acid. Free radical scavenging activity of hydrogen peroxide (%) was calculated as per formula (1).

Anthelminthic activity

The Anthelminthic activity of methanolic flower extract of *Nyctanthes arbour-tristis* was carried on *pheretima posthuma* (earthworms) (Mounika P, 2016). 20ml of 100mg/ml, 200mg/ml, and 300mg/ml methanolic extract of *Nyctanthes arbour-tristis* (test solutions) were prepared in distilled water and containing and transferred into three different petri dishes containing 5 earthworms in each one. 20 ml of 10 mg/ml concentration Piperazine citrate (Haque Rabiu, 2011) was used as reference standard. Distilled water was used as the control. Movements of earthworms were observed to note paralysis and death time. Paralysis time was considered where the movements of earthworms were stopped. When earthworms showed no movement either by vigorous shaking or by sprinkling hot water on earthworms. The results obtained were explained in tabular form.

RESULTS AND DISCUSSION

Methanolic extract of Nyctanthes arbour-tristis was found to possess antioxidant flavonoids as the chief constituents by chemical tests and assays. The methanolic extract of Nyctanthes arbour-tristis showed significant anthelminthic activity at all concentrations, but the maximum activity was observed at 300mg/ml.

CONCLUSION

The above findings of the present research revealed that Methanolic extract of *Nyctanthes arbour-tristis* possess significant antioxidant and Anthelmintic activity. Hence we advocate the researchers to investigate the plant extract for further half-life prediction, toxicological studies and herbal formulation etc.

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