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Original Article

ANTIOXIDANT ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT ON THE ROOTS OF *NYCTANTHES ARBORTRISTIS*

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ABSTRACT

Objective: To determine the antioxidant activity of *Nyctanthes arbortristis* (Family-Oleaceae).

Methods: The hydroalcoholic extract of the root of plant *Nyctanthes arbortristis* was taken into considerations to determine the phytochemicals present in it. The extracts of the roots were evaluated for antioxidant activity by using different *in vitro* model like Reducing Power Method and DPPH method.

Results: In the current investigation it has been found that the Pet. Ether and Hydroalcoholic extracts showed potent antioxidant activity by reducing power, as the concentration of the extracts increased, the absorbance was also increased correspondingly.

Conclusion: The hydroalcoholic extracts of this plant showed potent antioxidant activity against the standard drug (Kaempferol).

Keywords: Nyctanthes arbortristis, Phytochemistry, Antioxidant Activity

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INTRODUCTION

Free radicals are generally highly reactive and unstable compounds that can generate in the body during normal metabolic functions or it can form in the body from the external environmental sources such as environmental pollution and cigarette smoking. Antioxidants are the substances that can protect human bodies from oxidative damage of free radicals [1]. To balance the oxidative state, plants and animals maintain a complex system of overlapping antioxidants such as glutathione and enzymes (e. g. Catalase and superoxide dismutase) produced internally or the dietary antioxidants like vitamin A, vitamin C (ascorbic acid), β-carotene and α-tocopherol (a synthetic vitamin E) etc. Excessive production of free radicals can cause severe damage of cells and these may lead to various chronic diseases like cancer, stroke, heart disease, diabetes etc. Vitamins containing antioxidant are also helpful for the improvement of immune system functions [2]. Now a day's lot of researches is going on to establish the therapeutic efficacy on the basis of herbs and spices as antioxidant potentiality [3].

Nyctanthes arbortristis is one of the most useful conventional plants in India. The various parts of plant-like fruits, leaves, seeds, flowers, barks and stem have important phytochemicals and have some medicinal importance for treatment and management of different disease states. Phytochemicals such as flavonoids, oleanic acid, carbohydrates,saponins, tannic acid, carotene, lupeol, benzoic acid present in various parts of plant which have significant antiviral, antifungal, antipyretic, antihistamine, anti-malarial, antibacterial, anti-inflammatory, antioxidant activities On the basis of traditionally claim on *Nyctanthes arbortristis* plants and due to the lack of proper scientific investigation of their potential pharmacological properties, the main aim of this present study has to determine the antioxidant activity of the plant (*Nyctanthes arbortristis*)by using reducing power (RP), DPPH scavenging methods [4, 5].

Scientific classification

Biological Source: *Nyctanthes arbortristis*

Family: Oleacea

Kingdom: Plantae

Order: Lamiales

Genus: Nyctanthes

Species: N. arbortristis

Vernacular names: Night-flowering jasmine (English), Harsingar (Hindi), Shefali (Bengali), Sheali (Assamese).

Distribution of the plant

It is widely distributed in south Himalayan regions and southwards to Godavari. Each and every part of the plant has some medicinal value so that it is commercially available. It grows at sea level up to 1500 m altitude, within a range of rainfall patterns. Nyctanthes prefers a scheduled and semi-shady place to grow [6].

Materials and methods

The roots of *Nyctanthes arbortristis* was collected from the localities in Rangiya, kamrup, Assam during the month of January 2018. *N. arbortristis* were collected, shade dried, powdered manually and sieve through a no. 60 mesh sieve. About 100 gms of powdered roots are extracted with the solvents (petroleum ether and methanol) in Soxhlet Apparatus and then it was filtered by muslin cloth and evaporated to dryness by using Rotary Evaporator. The percentage yield of the extract was listed below in table 1:

Phytochemical screening of the extract

Phytochemical screening was carried out for petroleum ether and hydro-alcoholic extract of *N. arbortristis* for the presence of of different phytoconstituents like alkaloid, flavonoid, phenolics, carbohydrate, glycoside and proteins [7].

In vitro antioxidant study

Reducing power methods (RP)

The experiment was performed according to the method given by Oyiazu (1986)[8]. In this method the desired concentration of the extract (different conc.10-100µg/ml) suspended in distilled water, 2.5 ml of 0.2 M-phosphate buffer (pH6.6), and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) were added. The mixture was incubated at 50 °C for 20 min. followed by addition of 2.5 ml of TCA (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml

$FeCl_3$ (0.1% w/v), and the absorbance was measured at 700 nm against blank sample by using Kaempferol as a standard.

Table 1: Percentage yield of various extracts

Extract	% yield
Petroleum ether	6.3
methanol	8.9

Free radical scavenging activity measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH)

The experiment was performed according to the method given by Blois [9]. 0.1 mM solution of DPPH· was dissolved in ethanol to be prepared. 1 ml of this solution was added to 3 ml of each extract solution in water at different concentrations (4-500 µg/ml).

The mixture was shaken vigorously and allowed to stand at room temperature for 30 min.

Then the absorbance was measured at 517 nm using a UV-Vis spectrophotometer.

Lower absorbance values of the reaction mixture indicated higher free radical scavenging.

Activity by using Kaempferol as standard [10].

DPPH scavenging effect (%) = [(Ao − A1/Ao) × 100]

Where,

Ao was the absorbance of the control reaction

A1 was the absorbance in the presence of the sample at various concentrations.

Thiobarbituric acid method (TBA)

The experiment was done according to the method given by Chang *et. al* [11]. Here the same samples were used those were prepared for FTC method. To desired sample solution, 1.0 ml of 20% aqueous trichloroacetic acid (TCA) and 2.0 ml of aqueous thiobarbituric acid (TBA) solution were added. The mixture was placed in a boiling water bath and kept for 10 min. Then we have to cool the reaction mixture. After cooling, it was then centrifuged at 3000 rpm for 20 min. Absorbance of the supernatant layer was measured at 532 nm [12]. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay. Both methods (FTC and TBA) were used to describe antioxidant activity by percentage inhibition:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Pet. Ether and Hydroalcholic extract of *N. arbortristis* roots showed the presence of phenolic, steroids, flavonoids and glycosides (table 2).

***In vitro* antioxidant effect of Kaempferol, RP, TBA *and* DPPH scavenging determination of *N. arbortristis* roots**

In the current investigation, it has been found that the Pet. Ether and Hydro alcoholic extracts showed potent antioxidant activity by reducing power, as the concentration of the extracts increased, the absorbance was also increased correspondingly. But the Hydroalcoholic extracts showed more potent activity than the other extracts, as compared with standard substances (Kaempferol) (table 1). The lipid peroxidation of Pet. Ether, Hydroalcoholic extracts and Kaempferol was found to be 29.25, 32.12, 33.05 respectively (table 3). The *in vitro* free radical scavenging activity of *N. arbortristis* by Thiobarbituric Acid Method showed the potent percentage inhibition in case of hydroalcoholic extracts as compared to the other extracts. The *in vitro* free radical scavenging of Pet. Ether, Hydro-alcoholic extract and Kaempferol was found to be 33.05, 8.23, 30.31 respectively (table 2). The free radical scavenging by DPPH method also showed potent result at higher doses for hydroalcoholic extract. From the above findings, it can be concluded that the plant *N. arbortristis* showed the strong antioxidant property. The IC_{50} value showed good results for hydroalcoholic extracts of *N. arbortristis* in reducing power, TBA and DPPH methods (5.93, 6.16 and 14.81 respectively). The results for IC_{50} values for the standard Kaempferol was found to be 2.48 for the above three methods (table 4). The graphical representation of the above-mentioned activities for the various extracts are given in fig. 1, 2, 3 and 4 respectively.

Table 2: Results of preliminary phytochemical screening

Chemical test	Pet. ether	Hydroalcoholic
Alkaloid	-ve	-ve
Tannins	+ve	+ve
Saponins	+ve	+ve
Glycoside	+ve	+ve
Carbohydrates	-ve	-ve
Flavonoids	+ve	+ve
Proteins and amino acid	+ve	+ve
Phenolics	+ve	+ve

*(+ve) and (-ve) symbol indicates the presence and absence of respective plant constituents.

Table 1: *In vitro* Antioxidant activity of Kaempferol (Stand.), Pet. Ether, Hydro-alcoholic extract of *N. arbortristis* by RP method

❖ **Reducing the power method**

S. No.	Extracts	Activity	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
01	Kaempferol(Stnd.)		33.05±0.001	45.57±0.002	57.02±0.001	67.30±0.05	73.87±0.01
O2	Pet. Ether Extract	**Reducing**	12.44±0.020	21.10±0.018	28.02±0.022	35.15±0.012	43.02±0.039
03	Hydro alcholic Extract	**Power**	17.47±0.019	26.34±0.020	34.13±0.025	46.36±0.010	57.12±0.030

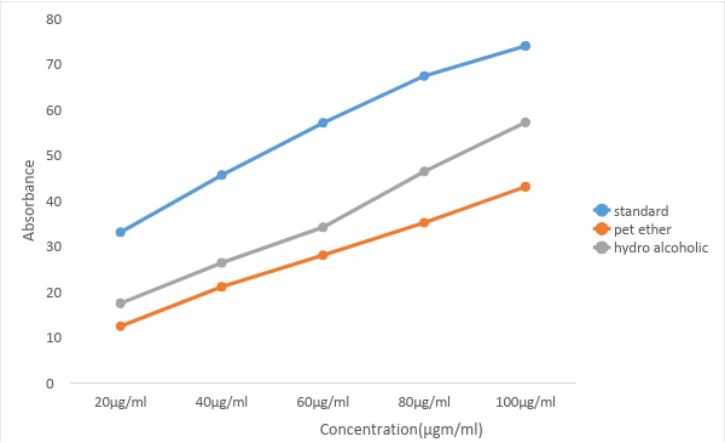

Fig. 1: Reducing power activity of different extracts of *N. arbortristis*

Table 2: *In vitro* antioxidant activity of Kaempferol (Stand.), Pet. Ether, Hydro-alcoholic extract of *N. arbortristis* by DPPH scavenging method

❖ **DPPH assay**

S. No.	Extracts	Activity	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
01	Kaempferol(Stnd.)		33.05±0.001	45.57±0.002	57.02±0.001	67.30±0.05	73.87±0.01
O2	Pet. Ether Extract	**DPPH**	08.23±0.12	14.49±0.012	15.06±0.003	18.01±0.032	21.12±0.43
03	Hydro alcholic Extract		30.31±0.32	35.47±0.017	42.06±0.014	47.51±0.032	54.32±0.43

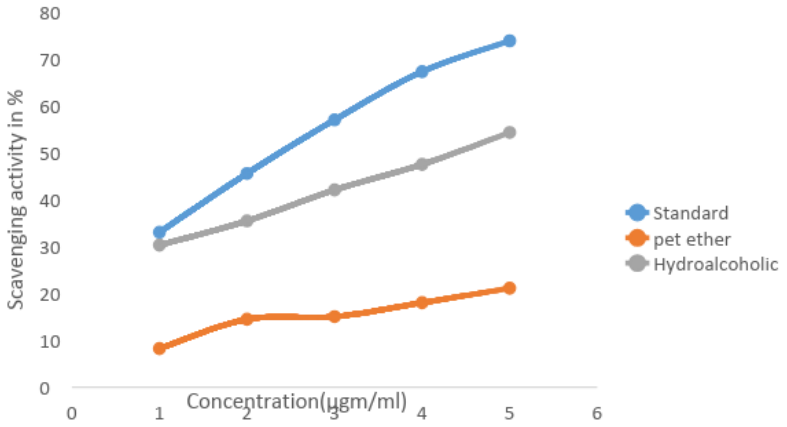

Fig. 2: DPPH scavenging activity of (% inhibition) *of N. arbortristis*

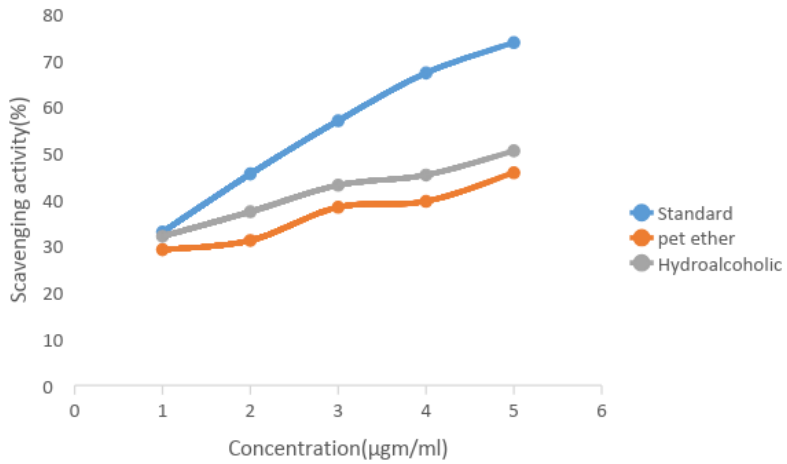

Fig. 3: TBA scavenging power activity (% inhibition) of *N. arbortristis*

Table 3: *In vitro* antioxidant activity of Kaempferol (Stand.), Pet. Ether, Hydro-alcoholic extract of *N. arbortristis* by TBA scavenging method

❖ **TBA method**

S. No.	Extracts	Activity	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
01	Kaempferol(Stnd.)		33.05±0.001	45.57±0.002	57.02±0.001	67.30±0.05	73.87±0.01
O2	Pet. Ether Extract	**TBA**	29.25±0.011	31.24±0.002	38.40±0.101	39.67±0.04	45.82±0.011
03	Hydro alcholic Extract		32.12±0.101	37.43±0.001	43.14±0.005	45.34±0.05	50.54±0.02

Table 4: IC_{50} value from reducing power, DPPH, TBA Scavenging activity of roots of *N. arbortristis*

S. No.	Activity	Extract	IC50
01	Reducing Power	Standard	2.48
		Pet. Ether	4.38
		Hydroalcholic	5.93
02	DPPH	Standard	2.48
		Pet. Ether	4.34
		Hydroalcholic	14.81
03	TBA	Standard	2.48
		Pet. Ether	4.85
		Hydroalcholic	6.16

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

There was no conflict of interest

REFERENCES

1. Santosh J, Manojkumar P. A review on nyctanthes arbortristislinn. Rejuvenating drugs. Int J Pharm Pharm Sci 2016;1:54-62.
2. Morel I, Lescoat G, Cogrel P, Sergent O, Pasdeloup N, Cillard P, *et al*. Antioxidant and iron-chelating activities of flavonoids cetechin, quercetin and diosmetin on iron-loaded rathepatocyte cultured. Biochem Phamacol 1993;45:13-9.
3. Solange IM, Lina FB, Silvia M, Jose AT. Extraction of antioxidant phenolic compounds from spent coffee grounds. Sep Purif Technol 2011;83:173-9.
4. Ondrejovic M, Kraic F, Benkovikiva H, Silhar H. Optimisation of antioxidant extraction from lemon balm (*Melissa officinalis*). Czech J Food Sci 2012;30:385-93.
5. Ashwini K, Beenu R, Tyagi V. Systemic review on anti-sciatica plant (Night Jasmine). Int J Curr Microbiol Appl Sci 2017;6:1018-35.
6. Divya P, Sheetal P. Phytochemical analysis of leave extracts of nyctanthesarbortristis. J Environ Sci Toxicol Food Technol 2015;1:39-42.
7. Ashwini K, Beenu R, Tyagi V. Systemic review on anti-sciatica plant (Night Jasmine). Int J Curr Microbiol Appl Sci 2017;6:1018-35.
8. Divya P, Sheetal P. Phytochemical analysis of leave extracts of nyctanthesarbortristis. J Environ Sci Toxicol Food Technol 2015;1:39-42.
9. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986;44:307-15.
10. Hanane E, Nadaya L, Katim A, Yahya C, Abdellah F, Brahim B, *et al*. Antioxidant activity, phytochemical screening, and total phenolic content of extracts from three genders of carob tree barks growing in Morocco. Arabian J Chem 2011;2:321-4.
11. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. J Agric Food Chem 2001;49:3420-4.
12. Ramazan A, Mehmet C, Ensari F. Protective effects of vitamin E, selenium and allopurinol against stress-induced ulcer formation in rats. Turk J Med Sci 2001;31:199-203.