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ORIGINAL ARTICLE

Antioxidant activity and Antifungal Activity of *Nyctanthes arbortristis*

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ABSTRACT

Ayurveda is one of the oldest medication systems that use plants and their extracts for treatment of various diseases. Nyctanthes arbor-tristis is an important shrub of tropical and subtropical regions in the world and here we communicate its antioxidant activity and antifungal activity. The antifungal activity ofdifferent parts (dried leaves, bark and flowers) of plant N. arbor-tristis was evaluated by preparing extracts in ethanol solvent. Their antifungal potential was measured by well diffusion method. Two most prevalent clinical pathogens Aspergillus Niger and penicillium were used as fungal specimens for this study. The ethanolic extract from leaves, and bark exhibited antifungal activity against respective fungus species. Theinhibition of A. Niger by ethanolic extract of N. arbor-tristis bark indicated higher potency. Ethanol extractof only leaves have also shown antifungal activity against A. Niger. The most significant antifungal activity is shown by ethanolic extract of bark. Antioxidants play a major role in curing degenerative disease such as cancer. In present time many synthetic antioxidants are commonly used but they have their toxic and carcinogenic effects, due to this reason their use has been restricted. So the research for natural antioxidants is very important. A study on natural compounds is of interest due to their satisfactory healthbenefits with low toxicity.

Key words: *Nyctanthes arbor-tristis, Antifungal, Antioxidant, Aspergillus Niger*

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INTRODUCTION

Free radicals are highly reactive molecules containing one or more unpaired electron; they donate or take electron from other molecule in an attempt to pair their electron and generate a stable species [1]. Antioxidants are both natural and synthetic compounds able to scavenge free radicals and inhibitoxidation processes [2]. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and used for industrial processing but they possess some side effect and toxic properties to human health therefore, there is an increasing interest in natural antioxidants, e.g. Polyphenols, present in medicinal and dietary plants [3]. Antioxidants play an importantrole in defending the body against free radical damage.

Recent studies have been shown that the leaves and stem of *N. arbor-tristis* possesses potential source ofnatural antioxidant [4].

The antioxidant activity of phenolic compound is mainly due to their redox properties, which can play animportant role in absorbing and neutralizing free radicals [5].

Nycatanthes arbor-tristis, is well known in India and its neighbouring countries as one of the most versatile medicinal plants. It is usually a shrub or small tree. *N. arbor-tristis* is

known as 'Night jasmine' (English) and 'Harshingaar' (Hindi) in India, due to the fact that its flowers emit a very strong and pleasant fragranceduring the whole night.
The generic name 'Nyctanthes has been coined from two Greek words 'Nykhta' (Night) and 'Anthos' (Flower) [6 ,7]. the specific name 'Arbortristis' meaning 'the sad tree' is derived from dull looks of the treeduring daytime.
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 [8, 9].

MATERIALS AND METHODS

CHEMICALS:
2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), ascorbic acid, methanol, n-Hexane, ferric chloride, nitric acid, sulphuric acid, potassium iodide, iodine, α-naphthol, sodium hydroxide, chloroform, hydrochloric acid allthe chemicals used were of analytical grade.

PLANT MATERIAL:
The leaves samples of *Nyctanthes arbortristis* were collected from the nursery in Agra region in the month of June then the samples were air dried in dark for 10 days and crushed them by grinder to form a fine powder and store inair tight container for further analysis.
The 10gm powder of leaves were extracted with the solvents such ethanol, at the same time 24hours by the Soxhlet extraction method. The extracts were filtered using the Whatman filter paper No.1. The filtrates were clean by the Column chromatography, the clean extract was concentrated to dryness under reduced pressure at 35^0C-40^0C using Buchi type vacuum rotator evaporator, the dry extracts obtained were stored in air tight container for the analysis.

TEST ORGANISMS:
The media used for antifungal test was Potato Dextrose Agar (PDA) with following composition Potato infusion - 200gm, Dextrose-20gm, Agar-15gm, distilled water-1000ml, pH7.4 at 25^0C. The clinical fungal test organism used for study are A. niger, A. flavus and Penicillium. The fungal strains are inoculated in PDA media for 24 hrs. Antifungal activity was shown by well diffusion method. Their antifungal potential was measured by well diffusion method in terms of zone of inhibition of fungal growth. All microorganismswere obtained from the stock cultures of the Department of Microbiology, Agra College, Agra.

DETERMINATION OF ANTIOXIDANT ACTIVITY:
Antioxidant activity is widely used as a parameter for medicinal bioactive components. Various methods are currently used to assess the antioxidant activity of plant. FRAP method were followed for determining the Antioxidant capacities of components. TPTZ (The FRAP assay (ferric reducing antioxidant power) evaluates total antioxidant power and is chosen to assess the presumable effects of medicinal plants.[10] FRAP assay depends upon the ferric tripyridyl triazine (Fe (III)-TPTZ) complex to the ferrous tripyridyl triazine (Fe (II)-TPTZ) by a reductant at low pH. Fe (II)-TPTZ (2, 4, 6-tri [2-pyridyl]-s-triazine) has an intensiveblue color and can be monitored at 593 nm [11].
The FRAP reagent was prepared by mixing solution A [300 mM acetate buffer prepared by dissolving sodium acetate (3.1 g/l) and glacial acetic acid (16 ml/l) in distilled water],

solution B [31.2 mg of 2,4,6- Tris(2-pyridyl)-s-triazine (TPTZ) dissolved in 10 ml of 40 mM HCl] and solution C [540 mg of ferric chloride hexahydrate dissolved in 100 ml distilled water] in a volume ratio of 10:1:1, respectively. The FRAP reagentwas warmed to 37 °C before being used. The assay was started by adding 1.8 ml of FRAP reagent to each well. Ascorbic acid used as a reference. Then the absorbance was taken at 593nm by the UV-Vis spectrophotometer (modal: Shimadzu UV-3600) and the ferric reducing antioxidant power determine bythe following equation:

$$\% \text{ of ferric reducing antioxidant power} = A_{sample} - A_{control} / A_{sample} \times 100$$

Where Abs_{sample} is the absorption of sample while $Abs_{control}$ is the absorption of TPTZ.

PHYTOCHEMICAL SCREENING:

The phytochemical screening of leaves extracts of *Nyctanthes arbortristis* is a preliminary step for determining the bioactive compounds present in the extracts. Phytochemical screening also plays a useful tool for the identification of compounds present extract. Following standard methods are used for the screening of these extracts–Wagner's test for Alkaloid's, Alkaline reagent's test for Flavonoid's, Salkowaski's test for Phytosterols etc.

RESULT AND DISCUSSION

ANTIOXIDANT ACTIVITY OF LEAVES EXTRACTS:

The antioxidant activities of these extracts were linearly increased with increasing concentrations (fig 1-2). The increasing orders of antioxidant activities of these extracts are similar to the reference compound ascorbic acid.

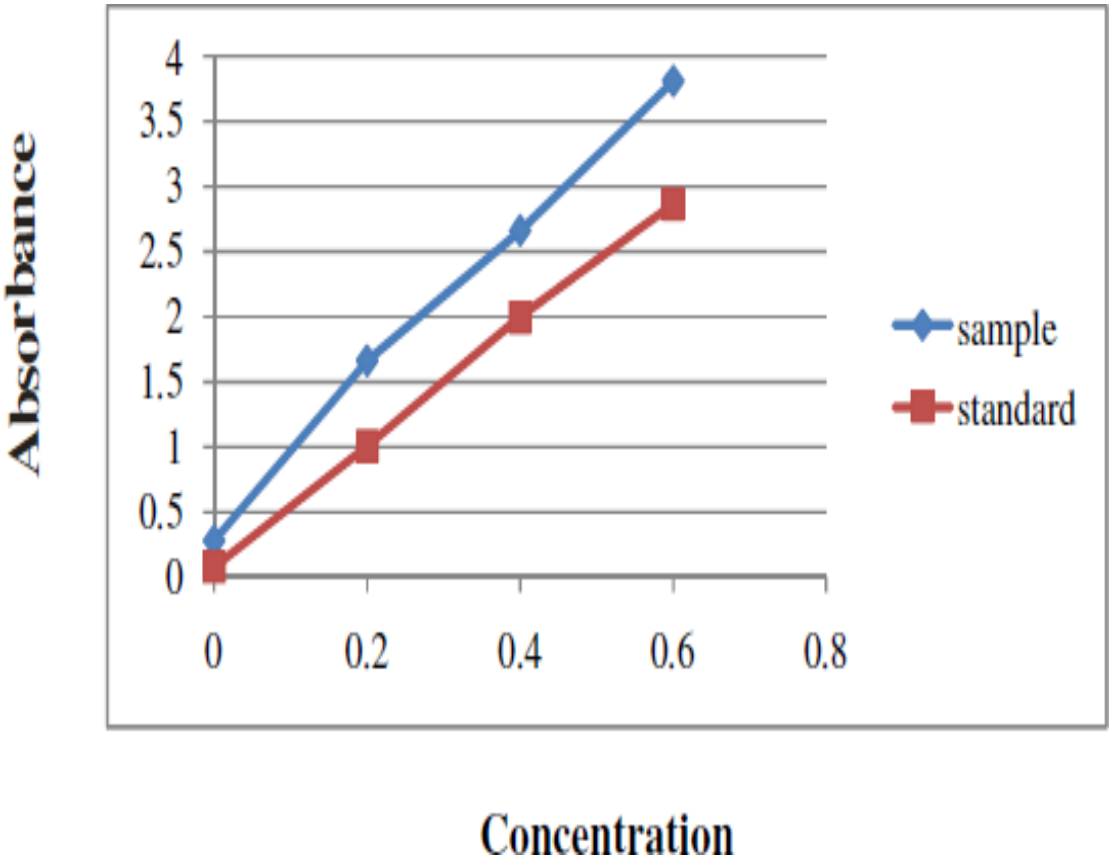

Fig 1: The standard linear curve: between absorbance against conc.

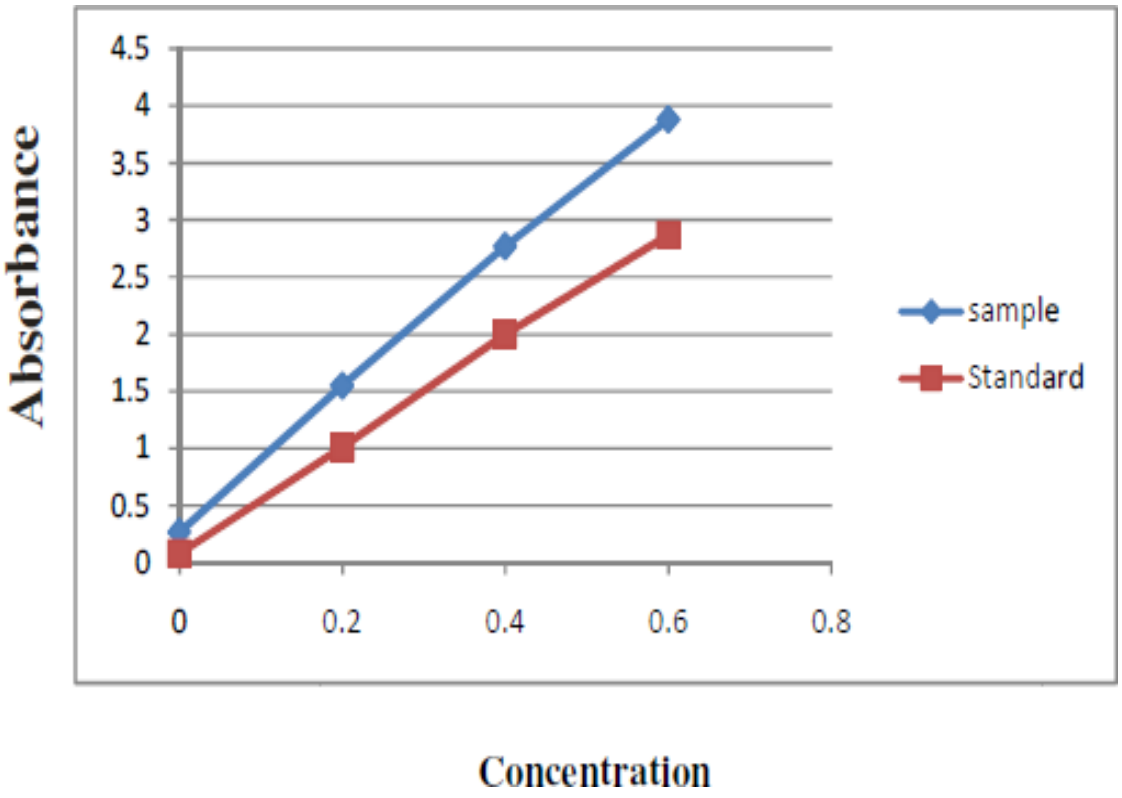

Fig 2: The standard linear curve: between absorbance against conc.

ANTIOXIDANT ACTIVITY OF BARK EXTRACTS:

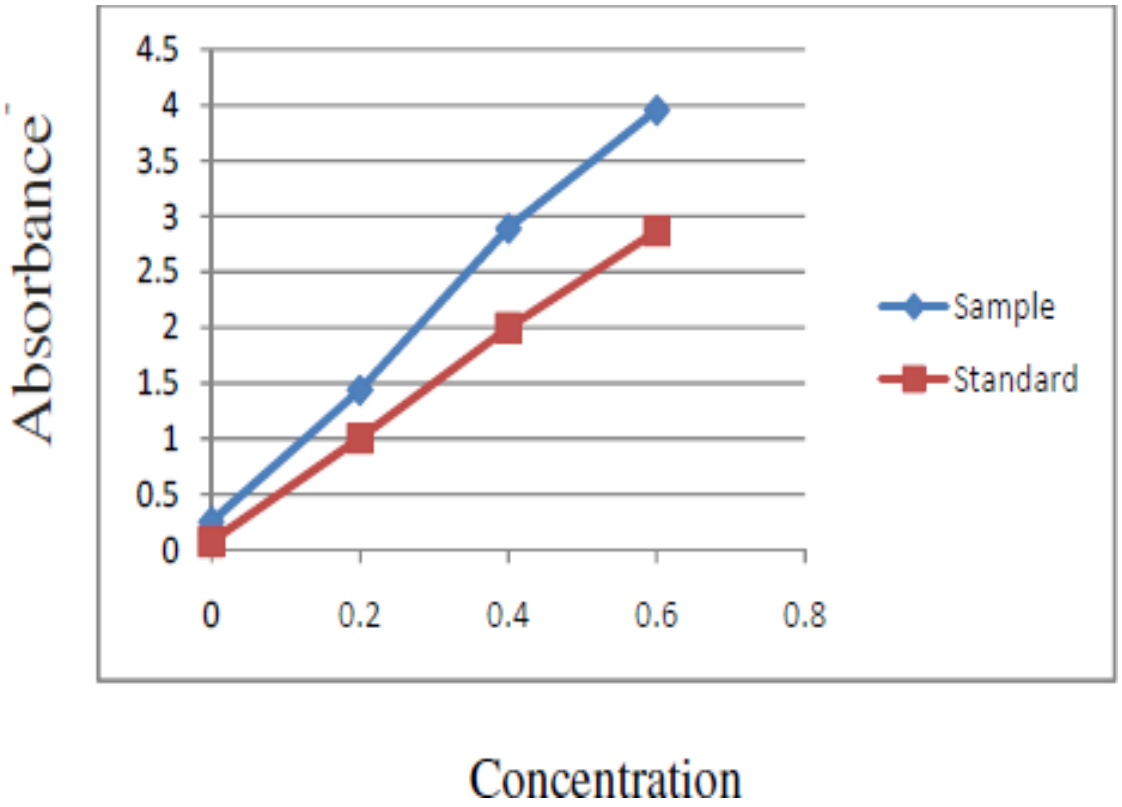

Fig 3: The standard linear curve: between absorbance against conc.

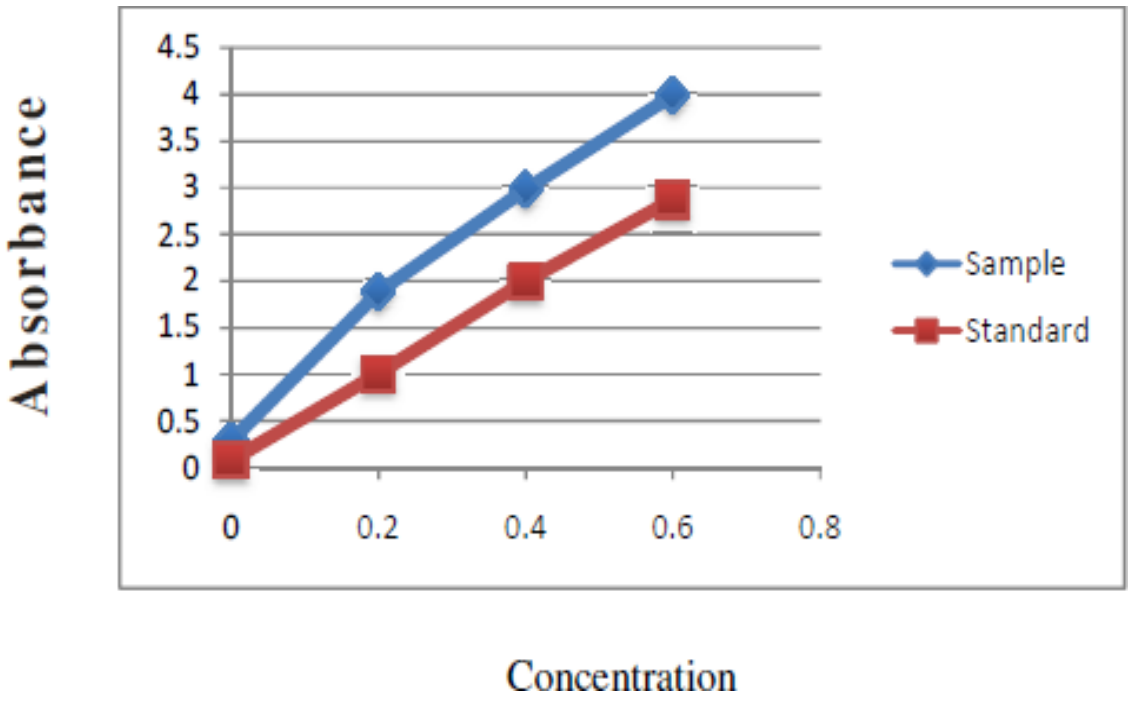

Fig 4: The standard linear curve: between absorbance against conc.

PHYTOCHEMICAL SCREENING OF LEAVES AND BARK EXTRACTS:

The phytochemical screening of leaves extracts (table 1) revealed that the alkaloids, phenols, protein, steroids,phytosterols and carbohydrate are present in both the extracts while terpenoids present only ethanolic leaves extract.

Table 1: Phytochemical screening of leaves and Bark extracts

	Alkaloids	Phytosterols	Flavonoids	Carbohydrate	Phenols	Proteins	Steroids	terpenoids
NLE	+	+	-	+	+	+	+	+
NBE	-	+	+	+	-	+	-	-

+ Present, - Absent

Where, NLE=*Nyctanthes* leaves ethanol extracts, NBE= *Nyctanthes* bark ethanol extract

ANTIFUNGAL ACTIVITY:

Identification of F. oxysporum

F. oxysporum grown on the PDA media produced white mycelia, with a cotton appearance that was pinkin colour on the underside of the plate. The microscopic features observed during identification were septations and shapes of conidia and chlamydospores. Masses of conidiophores were produced in the culture. The microconidia were abundant and kidney-shaped. The macroconidia were also abundant, slightly curved (sickle cell shape) with septations.

Antimicrobial activity of the plant extracts

The antifungal activities of *Nyctanthes arbortristis* leaves extracts and *Nyctanthes arbortristis* bark extracts against aspergillus niger were demonstrated by observable zones of inhibition. The mean inhibitory zones were highest at 100g/l in both plants,

although Nyctanthes flower extract portrayed the highest inhibitory activity (Table 2). Concentration at which no visible growth was observed considered asMIC.

Table 2: Mean inhibitory zones of *Nyctanthes arbortristis* leaves and *Nyctanthes arbortristis* bark in different concentrations

Treatment concentration g/l	Plant extracts	MIC in (mm)
100	*N. arbortristis* leaves extract (EtoH)	14.66
	N. arbortristis bark extract (EtoH)	9.33
50	*N. arbortristis* leaves extract (EtoH)	12.44
	N. arbortristis bark extract (EtoH)	7.12
25	*N. arbortristis* leaves extract (EtoH)	11.10
	N. arbortristis bark extract (EtoH)	5.11

A combined extract of the plants produced a slightly more antifungal activity against aspergillus niger compared to when the extract was used singly. The effect of the plant extract and the positive control was statistically significant ($p < 0.05$). The mean zone of inhibition of the plant extracts ratios ranged from 17.25 to 9.45 mm (Table 3).

Table 3: Zones of inhibition of *A. niger* when exposed to different ratios of the combined plant extracts of *Nyctanthes* leaves extract and *Nyctanthes* bark extracts

Plant Code	Combined Ratio (ml)	Mean zone of inhibition (mm)
	Control	22.68
NLE : NBE	0:30	7.34
	10:20	13.10
	30:30	15.12

The highest mean of inhibition was experienced in the combined extract where both plants had the same ratio. Treatments, where *Nyctanthes* leaves extract had the highest concentration, produced the highestzone of inhibition as compared to the treatment where *Nyctanthes* bark extract was the highest. However, there was a significant difference between the control and the combined plant extracts.

CONCLUSION

The results of the present study indicate clearly that the extracts from the leaves and bark of *Nyctanthes arbortristis* possess antioxidant properties, antifungal activity and could serve as free radical inhibitors or scavengers, which may act as primary antioxidants, specially ethanolic extract of leaves. Which can be used in drug development and pharmaceutical industry, and it is also a good source of antioxidants and useful drugs.

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