Original Article

In Vitro Antioxidant Activity Of Ethanolic Leaf Extract Of Nyctanthes Arbor-Tristis

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Abstract

The use of synthetic antioxidants has been restricted in the current scenario due to their hazardous and carcinogenic effects. As a result, a hunt for natural antioxidants is critical. Natural chemicals are of interest for research because of their safe and healthier implications and minimal toxicity. The aim of this study is to analyse the antioxidant potential of *Nyctanthes* leaves which may be attributed to large amounts of phenolic constituents present in the leaves of this traditionally valuable plant. The ethanolic extract of the leaves of *Nyctanthesarbor-tristris* was tested by *in vitro* antioxidant method using DPPH (1,1-diphenyl-2-picrylhydrazine).The extract was taken in five different concentrations ranging from 100-500 µg/ml. The results obtained were comparable to the standard ascorbic acid. This alludes that *Nyctanthes* leaves can be used as a potential source of free radical scavenging activity.

Keywords – Antioxidant, DPPH, Ascorbic acid, Free radical scavenging activity, *Nyctanthes* leaves, Ethanolic extract.

INTRODUCTION

The reactive oxygen species such as singlet oxygen, superoxide and hydroxyl radicals (1,2,) are continuously produced in the living bodies as a result of various undesirable environmental factors. The accumulation of these free radicals may be a source of diseases like Cancer, Heart diseases, Diabetes mellitus, Aging and Inflammatory conditions (3,4). *Nyctanthesarbor-tristris* (Nyctanthaceae) (5,6) is widely available as a medicinal plant all over India. It even grows in wild in the southern states of the country. The plant is known to possess phytoconstituents such as alkaloids, carbohydrates, phenols, and flavanoids glycosides and thus it finds a large number of uses in Ayurveda, Siddha and Unani systems of medicine. Phytochemical studies on various plant parts such as flowers, leaves seed, bark and fruits have shown the presence of triterpenes (lupeol, β-amyrin, oleanic acid), alkaloids(nyctanthine), flavanoid glycosides, β-Sitosterrol, tannic acid, mannitol, glucose, fructose, ascorbic acid, methyl salicylate and benzoic acid (7,8,9). The leaves have been reported to be useful as expectorant diuretic, laxative, diaphoretic (10, 11, 12), in sciatica, rheumatism and spleenomegaly(13,14,15).

Aqueous and alcoholic extracts of the leaves are also used against intestinal worms (16), Antileishmanial (17,18). Extracts of the leaves have exhibited inhibition of writhing reflex in rats, also both the extracts have shown activity against tail immersion test (19). Hepatoprotective, antifugal, antiviral, immunosimulant activities have also been reported(20, 21, 22,23). The leaves have also been traditionally useful in bronchitis, constipation, and baldness, skin diseases constipation, ulcer(24,25), also as digestive, analgesic(26) and in gynaecological problems(27). Decoction of the leaves have been traditionally used in malaria, as a hair tonic and in alopecia (28,29,30,31). Different parts of Nyctanthes are used by tribes of Central India for snakebite, dysentery (32) andantiallergic(33),amoebicidal (34) and anthelmintic (35) . The leaf extract has also been used in treating Hemorrrhoid, Pyrexia and biliary disorders with leaf succulent of Nyctanthes(36).The presence of flavanoidal glycosides like nicotiflorine and astragline and phytoconstituents like Iridoid glycosides (arborsides A, B and C), 6β-hydroxy loganin(37) may contribute to the free radical scavenging activity (38, 39,40) hence, it was decided to study the antioxidant activity of the ethanolic extract using the DPPH method(41).

MATERIALS AND METHOD

Chemicals And Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemicals Co., USA. Ascorbic acid and all other chemicals and solvents used were of analytical grade.

Collection Of Plant Material

The leaves of *Nyctanthesarbor-tritris* were purchased from Ayush Life Elements,8,Pustak Bajar, Neemuch, Madhya Pradesh and authenticated by Dr. Sunita Garg, former Chief Scientist Head, RHMD,CSIR-NIScPR, New Delhi (taxonomic reference number: NIScPR/RHMD/4041-42).

Preparation Of Extract

The dried leaves were made into powder form. 125 g of the powedered drug was extracted with 700 ml of ethanol in a Soxhlet apparatus. After complete extraction process, the extract was concentrated using a rotary evaporator at 40ºC. The dried extract was used for further study.

Antioxidant Assay

DPPH Radical Scavenging Assay

The free radical scavenging activity was measured *in vitro* using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to the method reported by (42). 0.1 mM solution of DPPH was prepared and 1 ml of the DPPH solution was added to 3 ml of the leaf extract. Five different concentrations ranging from 100-500 µg/ml of the ethanolic extracts were used. The mixture was shaken vigorously and incubated for 35 mins in the dark at room temperature. When DPPH reacts with antioxidants in the sample, it gets reduced and its colour changes from deep violet to light yellow. The absorbance was measured at 517 nm using UV-visible spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula:

(%) Inhibition = [(Absorbance of control–Absorbance of sample)/(Absorbance of control)] ×100.

RESULT AND DISCUSSION

The ethanolic extract of *Nyctanthes arbor-tristis* leaves was assessed for its DPPH radical scavenging activity in comparison to standard ascorbic acid in this research. The ability of *Nyctanthes arbor-tristis* ethanolic leaf extract to scavenge free radicals was demonstrated by DPPH bleaching. Table 1 shows that the ethanolic leaf extract of *Nyctanthes arbor-tristis* had the maximum antioxidant activity of 61.44% when compared to standard ascorbic acid, which had an antioxidant activity of 63.92% (figure 1).The study indicates that the extract has proton donating abilities, proposing that it could be used as a free radical inhibitor or scavenger.

CONCLUSION

The antioxidant activity of an ethanolic extract of *Nyctanthes arbor-tristis* leaves was assessed *in vitro*. When compared to standard ascorbic acid, the DPPH assay findings demonstrate that the extract possesses antioxidant activity, indicating that this leaf extract could be a substantial source of natural antioxidants that could help prevent diseases related with oxidative stress.

Table 1: DPPH radical scavenging activity of ethanolic extract of *Nyctanthes arbor- tristis*leaves

S.No.	Concentration (µg/mL)	% Inhibition	
		Ascorbic Acid	**Sample**
1	100	51.48	44.21
2	200	56.86	49.68
3	300	58.54	54.65
4	400	60.17	58.35
5	500	63.92	61.44

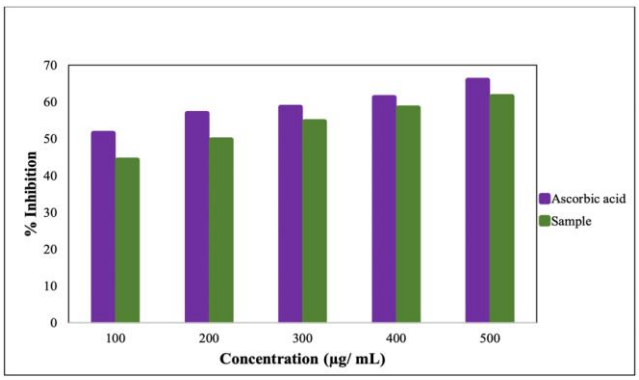

Figure 1: Percentage of free radicals scavenged by ethanolic extract of *Nyctanthes arbor- tristis* leaves using Ascorbic acid as standard (DPPH free radical scavenging method)

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