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In vitro free radical scavenging activity of *Nyctanthes arbor-tristis* L. leaf extracts

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Abstract
The present study was carried out to evaluate and find the extracts having good *in-vitro* antioxidant properties from *Nyctanthes arbor-tristis* L. (Parijat). An *in-vitro* free radical scavenging activity of the different extracts was using DPPH (1, 1-Diphenyl-2-picrylhydrazyl) method. Methanol, chloroform and aqueous extracts of plant were prepared by soxhlet extraction and used to evaluated antioxidant property. The different concentration of the extract (µg/mL) showed good free radical scavenging property which was calculated as a percentage inhibition. The results showed that highest percentage inhibition of the methanol, chloroform and aqueous extracts were found to be 66.38, 32.90 and 37.93 % at 50 $\mu g.mL^{-1}$ against DPPH activity, respectively. The extracts showed significant antioxidant activities compared to the ascorbic acid. Methanolic extract of *N. arbor-tristis* showed good inhibition when compared to the reference standard ($P<0.05$) which might be responsible due to high phenolic content in the leaf extract. It was concluded that methanol extract of *N. arbor-tristis* leaf exhibited potent free radical scavenging activity.

Keywords: scavenging activity, percentage inhibition, Methanol

Introduction
Free radicals or reactive oxygen species (ROS) are continuation produced by metals, environmental factors and air pollution in living organism demolishing the biochemical process at cellular level [1]. Free radicals are known to be the source of major causes of various human and animal diseases like chronic and degenerative diseases including aging, coronary heart disease, stroke, diabetes mellitus, cancer and inflammation [2]. Now day searching natural antioxidant are important source of medical plants. Secondary metabolites like's phenolic, tannins, flavonoids are the antioxidants properties which can prevent various biochemical processes invade free radicals by saturating lone pair of electron bears by ROS. Anti-oxidant agents may be use as a preventive or therapeutic agents against various disease condition [3].
Nyctanthes arbor-tristis L. commonly known as night jasmine or 'Parijat' belongs to the family Oleaceae. It is widely cultivated all over India as a garden/medicinal plant and also found in the forest of South Indian areas. It is commonly used in the traditional system of medicines in the treatment of chronic fevers, rheumatism, biliary disorders chronic fever, and malarial fever. It has immense therapeutics properties. The indigenous people of India use *Nyctanthes arbor-tristis* to cure various ailments along with its use in Ayurveda, Siddha and Unani systems of medicines [4]. The reported phytochemical are arborsides A, B, C, flavonol glycosides, astragalin and nicotiglorin present in various parts of plant which have significant hepatoprotective, antiviral, antifungal, antipyretic, antihistamine, anti-malarial, antibacterial, anti-inflammatory, antioxidant activities [5]. Flavonoids are large group of compounds occurring abundantly in plants. They occur as glycosides and contain several phenolic hydroxyl groups on their ring structure. Many flavonoids are found to be strong free radical scavengers and antioxidants. Thus, present study was planned to screen different extracts of *Nyctanthes arbor-tristis* L. leaf for having *in-vitro* antioxidant activity.

Materials and Methods
Collection of plant material: *Nyctanthes arbor-tristis* L. leaf were collected from surrounding of Junagadh district and authenticated by Botanist.

Preparation of extract: Different extracts of this plant was prepared by soxhlet extraction method. About 30 gm of powdered material was uniformly packed into a thimble and run in

soxhlet extractor. It was exhaustible extracted with chloroform, aqueous and methanol for 72 hours. After that extracts were filtered with the help of filter paper (Whatman No. 1) and solvent was evaporated in a rotary evaporator to get extract which was used to evaluate *in-vitro* anti-oxidant activities.

Phytochemical screening of *Nyctanthes arbor-tristis L. leaf*

Qualitative phytochemical screening of different extracts *Nyctanthes arbor-tristis L.* leaf was carried out for presence of alkaloids, flavonoids, saponin, phenols, sterol, protein and tannins as per standard methods [6].

Antioxidant activity: The scavenging activity of *Nyctanthes arbor-tristis* L. leaf extracts was determined using DPPH assay [7]. 1mg per mL solution was prepared by dissolving 30 mg extract in 30 mL Milli-Q water. Chloroform extract was dissolved by incorporating 5 to 10% DMSO as a solvent. Suitable dilutions were made from this stock solution with Milli-Q water only.

All the dilutions were taken in test tubes up to 3 mL of sample solution of different concentrations (10, 20, 30, 40, 50 µg/ml). 1 mL of 0.1mM DPPH solution was added. Solution was kept for 30 minutes at room temperature until color changed from violet to yellowish violet to yellow. Blank solution containing distilled water and DPPH alone (Control) were also prepared and spectra were recorded for control purpose. Ascorbic acid was used as positive control and prepared in the same manner as above. The absorbance values were measured at 517 nm and converted into the percentage antioxidant activity using the following equation. The entire assay were performed in triplicates and repeated three times at different time points and the absorbance was presented as mean ±SE.

$$\%\ Inhibition = \frac{Absorbance of Control - Absorbance of Test}{Absorbance of Control} \times 100$$

Apart from antioxidant activity, total phenolic content was also measured by Folin Ciocalteu method (Encarnacão *et al*., 2015). In this assay, 250µl of extract solution were allowed to react with 2milliliter Folin-Ciocalteu (FC) reagent (previously diluted 1:10) and1 milliliter solution (75g/L) of sodium carbonate. The mixture was allowed to stand for 1 to 2 hours. Absorbance was recorded at 760 nm in spectrophotometer. Tests were performed in triplicate. Total phenolic content (TPC) was expressed as milligrams of gallic acid equivalents (GAE).

Statistical analysis: All the data were expressed in mean ± S.E. (n=3). Selected data were analyzed by one-way ANOVA followed by Duncan Multiple Range Test (DMRT) to compare difference in means.

Results and Discussion

Presence of various qualitative phytochemicals screening of *Nyctanthes arbor-tristis* L. leaf extract are shown in table 1 which revealed the presence of carbohydrate, flavonoids, alkaloid, phenols, tennins and glycosides. Results of present study were in support of Divya Paikara *et al*., 2015 [9]

In study antioxidant activities of *Nyctanthes arbor-tristis* L. leaf extract of methanol, chloroform and aqueous were performed by i*n-vitro* DPPH scavenging effect. The total phenolic content of aqueous and methanol extracts were found to be 238 and 372 µg/100g Gallic equivalent/gram extract, respectively. Phenolic compound are one of the most important groups of secondary metabolites. Most pharmacological activities reported from *Nyctanthes arbor-tristis* L. leaf extract may due to flavonoids and high phenolics (like flavanol glycosides and astragalin) contents could be responsible for the antioxidant potential [10].

The maximum % inhibition on DPPH radical scavenging activity was 66.38 (Methanol), 32.90 (Chloroform), 37.93 (aqueous) and 64.66 (ascorbic acid) at a concentration of 50 µg/ml. However, a maximum inhibition all extracts of plant were compare to ascorbic acid. [Table 2]. Antioxidant activity of methanol extract of *Nyctanthes arbor-tristis(L)* leaves was found higher percentage inhibition at concentration of 50 µg/ml which might be responsible due to presence of glycosides, tennins and flavonoids. Many studies supports our result that *Nyctanthes arbor-tristis* L possess anti-oxidant activity [11-13]. Natural antioxidants is potential source of *Nyctanthes arbor-tristis* L Leaf. Secondary metabolites of different extract leaf *Nyctanthes arbor-tristis* L contain phenolic compound, tannin and flavonoids that have been found good antioxidant agent. Leaf extracts of *Nyctanthes arbor-tristis* L. produced the very strong inhibition of DPPH.

Conclusion

Methanol extract from *Nyctanthes arbor-tristis* L leaves produced very good inhibition against free radicals was due to high phenolic content in the leaf extract. Many flavonoid and secondary metabolites from crude extract of plant and showed effective anti-cancer, hepatoprotective, antiviral, antifungal, antipyretic, antihistamine, anti-malarial, antibacterial, anti-inflammatory and antioxidant activities The DPPH assay is a rapid screening and economic method, which has been commonly used for the evaluation of the *in-vitro* anti-oxidative properties of medicine plants. The result of this study show that methanol extract of *Nyctanthes arbor-tristis (L)* leaves was found major source of natural antioxidant.

Table 1: DPPH scavenging activity of various extracts of *Nyctanthes arbor-tristis* L. leaf

Concentrations µg/ml	Percent inhibition (mean ±SE)			
	Ascorbic acid	Chloroform	Methanol	Aqueous
10	50.72±3.24^{c}	6.61±3.77^{a}	45.69±1.51^{b}	9.20±2.95^{a}
20	57.18±4.05^{d}	16.09±6.25^{a}	53.45±2.59^{c}	20.98±4.17^{a}
30	59.77±2.54^{d}	22.84±5.50^{a}	60.20±3.11^{d}	31.18±4.99^{a}
40	63.94±1.66^{e}	27.44±4.94^{a}	63.51±2.86^{e}	36.35±2.91^{a}
50	64.66±2.04^{e}	32.90±6.32^{a}	66.38±2.04^{e}	37.93±1.32^{a}

Values in the same column with different superscripts are significantly (P<0.05) different.

Table 2: Phytochemical screening of leaf extracts of *Nyctanthes arbor-tristis* L.

Sr. No	Phytochemicals constituents	Tests	Extracts	Result
1	Alkaloid	A. Mayer's test	Water	Negetive
			Methenol	Negetive
			Choloroform	Negetive
		B. Drangendorff's test	Water	Negetive
			Methenol	Negetive
			Choloroform	Negetive
		C. Wagner's test	Water	Negetive
			Methenol	Negetive
			Choloroform	Negetive
		D. Hager's test	Water	Negetive
			Methenol	Negetive
			Choloroform	Negetive
2	Flavonoid	a. Shinoda test with magnesium metal	Water	Positive
			Methenol	Positive
			Choloroform	Negetive
		A. Shinoda test with zink metal	Water	Positive
			Methenol	Positive
			Choloroform	Positive
3	Saponin		Water	Positive
			Methenol	Negetive
			Choloroform	Negetive
4	Sterol	Salkowaski's test	Water	Positive
			Methenol	Positive
			Choloroform	Negetive
5	Glycosides	A. Molisch's test	Water	Positive
			Methenol	Positive
			Choloroform	Positive
		B. Sulfuric acid test	Water	Positive
			Methenol	Positive
			Choloroform	Negetive
6	Tennins	A. Ferric chloride test	Water	Positive
			Methenol	Negetive
			Choloroform	Negetive
		B. Lead acetate test	Water	Positive
			Methenol	Positive
			Choloroform	Positive

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