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Comparative Evaluation of Antioxidant Activity of Aqueous and Methanolic Extract of Nyctanthes arbortris-tis Linn. by Using DPPH Assay

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***Abstract*: *Nyctanthes arbortristis* is one of the useful traditional medicinal plants in India. It is commercially exploitable because each part of the plant has some important medicinal value. It is now considered as a valuable source of several unique products for the medicines against various diseases and also for the development of some industrial products. The present study is to focuses on the comparison of antioxidant potential of aqueous & methanolic extract of plant *N. Arbortristis*. The antioxidant assay was performed using DPPH scavenging activity and ascorbic acid was used as positive control & water was used as negative control. The observations were recorded at 517nm. & the results showed maximum antioxidant activity in the methanolic extract when compared with aqueous extract.**

***Keywords*: Nyctanthes arbortris-tis Linn., Antioxidant.**

1. Objective

To perform & compare Antioxidant activity of the aqueous and methanolic extracts of leaves of *Nyctanthes arbor-tristis* L. using DPPH assay.

2. Introduction

Fig. 1. Nyctanthes arbor-tristis L. plant

*N. arbortristis*is a valuable traditional medicinal plant which belongs to the family Oleaceae. It is usually a shrub or a small tree having brilliant, highly fragrant flowers, which bloom at night and fall off before sunrise, giving the ground underneath a pleasing blend of white and red. The plant generally grows in tropical and subtropical region. Thus, during the day the plant loses all its brightness and hence is called "Tree of sadness" Medicinal plants containing steroids, alkaloids, glycosides, insecticide, additives and related active metabolites are of great value in the drug and pharmaceutical industry.

Fig. 2. Leaves of N. arbortristisis

Table 1
Classification of Plant

Class	Eudicots
Division	Angiosperm
Family	Oleaceae
Genus	Nyctanthes
Kingdom	Plantae
Order	Lamiales
Species	Nyctanthes arbortristis

A. Morphological characters

N. arbortritis is light to light green in colour. It has indistinct odour. It is bitter and astringent in taste. Leaves are Simple, 5-14 cm long, 2.5-7.5 cm wide, ovate, acute to acuminate; both surfaces rough, scabrous. Margin is entire or distinctly toothed. It has round to somewhat cuneate base. Venation are reticulate, lateral vein 3-6 pairs more conspicuous on lower side.

B. Phyto-constituents of N. arbortritis

Bark contains Alkaloids, Glycosides. Flower oil contains Apigenin, Anthocyanin, D-Mannitol, Tanninm, Glucose,

Carotenoid, Essential Oil, Kaemferol, Nyctanthin, Glycosides, Quercetin, Rengylone, α-crocetin (or crocin-3), β-monogentiobioside, β monogentiobioside-β-D, β digentiobioside. Leaves contains Apigenin, Anthocyanin, D-Mannitol, Tanninm, Glucose, Carotenoid, Essential Oil, Kaemferol, Nyctanthin, Glycosides, Quercetin, Rengylone, α-crocetin (or crocin-3), β monogentiobioside, β-monogentiobioside-β-D, β-digentiobioside.3-4 Secotriterpene Acid, a Pale Yellow Brown Oil (15%), Arbortristoside A & B, Glycerides of Linoleic Oleic, Lignoceric, Myristic Acids, Nyctanthic Acid, Palmitic, Stearic these all constituents are obtained through seeds. And stem contains constituents such as Glycoside-naringenin-4'-0-β glucapyranosyl-α-

Xylopyranoside, β sitosterol.

3. Plant Collection and Extraction Method

A. Plant collection

Fresh leaves of *N. arbortristis* was collected. It was washed using tap water & then using distilled water. Leaves were dried for overnight. Later leaves were placed in a tray and was dried using a Tray drier at 55^0c for about 2 hours. Then leaves were packed in a polythene bag and placed in a tight container.

B. Powder preparation

Dried leaves were first crushed in a polyethene bag by using hands and further grinded into powder using a mixer grinder. Powdered leaves were sieved using sieve no.12. Then powder was stored in a self-sealable bag.

Fig. 3. Fresh leaves of Nyctanthes arbor-tristis L.

C. Extraction method

1) Aqueous extraction

Procedure: A conical flask was filled with 250ml of water and accurately weighed 30gm of leave powder was mixed in the flask. It was placed on a magnetic stirrer for 15 hours. Aqueous extract was separated using vaccum filteration (Buchner funnel). Extract was concentrated by direct heat and then dried on a Hot plate. A brown coloured powder was obtained.

2) Methanolic extraction

Procedure: Soxhlet apparatus was filled with accurately weighed with 60gm of powdered leaves. Then RBF was filled with 150 ml Methanol and fitted with the soxhlet apparatus. 100ml of methanol was added to the thimble directly. The soxhlet extraction was placed on a heating mantle at 45-50oc for about 7 hours. Extract was then filtered with sintered glass filter. The extract was concentrated using directly heated. A dark green extract was obtained.

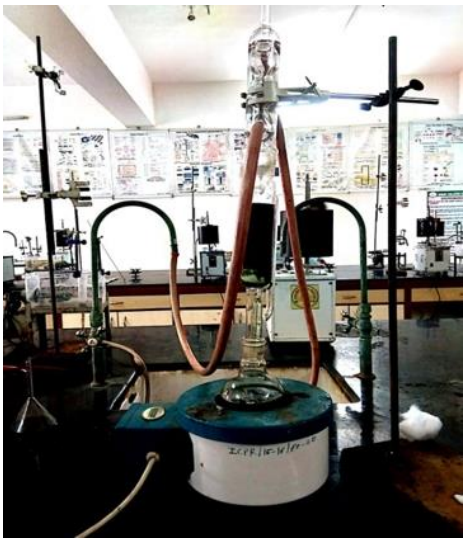

Fig. 4. Soxhlet apparatus for methanolic extraction

Fig. 5. Aqueous extraction

D. Antioxidant Activity

The scavenging activity of Nyctanthes arbor-tristisL. leaf extracts were determined using DPPH assay.

Procedure: 1mg per mL solution was prepared by dissolving 50 mg extract in 50ml deionized water. Suitable dilutions were made from this stock solution with deionized water. 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. Solutions kept for 30 minutes at room temperature. Color changed from violet to yellowish violet to yellow. Blank solution was prepared with distilled water and DPPH alone (Control) and spectra is recorded. Ascorbic acid was used as positive control and prepared in the same manner as above. Absorbance values measured at 517 nm. Converted into the percentage antioxidant

activity using the following equation. The entire assay was repeated three times at different time interval and this absorbance are presented as mean.

Formula to calculate Antioxidant effect:

Antioxidant effect (%) = A_C-A_S/A_C x 100

(Where, A_C = Control absorbance, A_S = Sample absorbance)

Observation table:

Recorded under UV spectrophotometer at a wavelength of 517 nm.

Table 2
Observation table

Concentrations µg/ml	Absorbance at 517 nm (Mean)		
	Ascorbic acid	Methanolic extract	Aqueous extract
10	0.4361	0.3729	0.7914
20	0.4071	0.3049	0.7495
30	0.3846	0.2793	0.5781
40	0.3021	0.2749	0.5434
50	0.2948	0.2681	0.5399
Control absorbance = 0.8739			

% Antioxidant activity:

Table 3
% Antioxidant activity

Concentrations µg/ml	Antioxidant effect (%)		
	Ascorbic acid	Methanolic extract	Aqueous extract
10	50.09	57.32	9.4
20	53.41	65.11	14.23
30	55.99	68.03	33.84
40	65.43	68.54	37.81
50	66.26	69.32	38.21

4. Discussion

The scavenging activity of aqueous and methanolic extract of *Nyctanthes arbor-tristis* L. Leaf extracts is discussed below:

The maximum % antioxidant activity shown by ascorbic acid was 66.26% at 50 µg/ml. The maximum % antioxidant activity shown by methanolic extract of *Nyctanthes arbortristis*L. leaf was 69.32% at 50 µg/ml. The maximum % antioxidant activity shown by aqueous extract of *Nyctanthes arbortristis* L. leaf was 38.21% at 50 µg/ml. Compairing the % antioxidant activity between aqueous & methanolic extracts of *Nyctanthes arbor-tristis* L. leaf confirms that the methanolic extract shows more potent antioxidant activity as its readings are 65.11%, 68.03%, 68.54% & 69.32% at 20, 30, 40 & 50µg/ml.

5. Conclusion

The present study suggested that the methanolic extract of *N. arbor-tristis* leaf possess various phytochemical compounds having antioxidant potential which may be used for the oxidative stress related conditions and further investigation related to the active principle isolation and characterization may lead to newer chemical entities for clinical use or combinatorial synthesis. The replacement of synthetic with natural antioxidants (because of implications for human health) may be advantageous. It may provide nature friendly and cost effective drugs.

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