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THE INVESTIGATION ON TOTAL PHENOLIC CONTENT AND *IN VITRO* ANTIOXIDANT POTENTIAL OF DIFFERENT PLANT PARTS OF *NYCTANTHES ARBOR-TRISTIS* (NIGHT JASMINE)

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ABSTRACT: *Nyctanthes arbor-tristis* (Paarijaat) is one of the important plants used in traditional medicines, mainly known for the medicinal properties of its flower and leaf. In this study, the four plant parts of *Nyctanthes arbor-tristis* were extracted sequentially with four solvents of decreasing polarity and investigated for antioxidant potential. The highest phenolic content was observed with flower, followed by stem, primarily by aqueous and methanolic extracts. The methanol and ethyl acetate extracts from all four plant parts showed decent antioxidant activities against DPPH free radicals, with methanolic extract of *N. arbor-tristis* stem showed activity as 86.9%, higher than the positive control. All the ethyl acetate extracts showed moderate to good activity (50-65%), showing that all the four plant parts have decent antioxidant potential. Further, the scavenging of the peroxide free radicals, produced during lipid per oxidation was also investigated. The methanolic extracts of flower and root, and ethyl acetate extract of stem showed almost 80% activity. The aqueous extract of leaf showed very good peroxide scavenging activity. The hexane extract of leaf showed the highest peroxide scavenging activity. The study is one step forward to establish traditional uses in modern contexts.

INTRODUCTION: Among all ancient civilizations, plants were the source for almost all necessities, including medicines. A variety of native plants and plant parts were investigated over a period of more than 3000 years in order to get the optimum benefit from locally available plants. The treasure of this knowledge has emerged in various medicinal systems *i.e.* Ayurveda, Homeopathy, Greek, traditional Chinese, etc. As the science progressed, allopath came into the picture which is more or less an amalgam of various traditional practices with modern day science.

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In this new era of medicine, where a hunt for new drugs, especially of herbal origin is on, there is a need to reinvestigate the traditional medicinal plants with modern day techniques and approach to discover new drugs or to enhance the potency of existing formulations. *Nyctanthes arbor-tristis* is one of the jewels of traditional Indian medical science and this native plant of India is used in various diseases, as well as other purposes like perfumes and decorations. Mainly, known for its fragrant flowers, the other plant parts are also well described for their medicinal properties [1].

Apart from the usual medicinal properties *i.e.* antipyretic, antibacterial, hepatoprotective and anti-inflammatory activities [2]; antioxidant activity is more pronounced and various researches have been directed towards free radical scavenging potential of *N. arbor-tristis* [3]. Although, there are various reports that deal with antioxidant potential of *N.*

arbor-tristis; but the focus has been given mainly to the flowers [3], and to some extent, leaves [4]. A comparative account of all the plant parts is the need of the time to characterize the pharmacological activities of different plant parts obtained at the same time and from the same geo-climatic zones to get deeper insight.

MATERIALS AND METHODS:

Collection and Preparation of Plant Material: The *Nyctanthes arbor-tristis* (in Hindi, "Parijaat"), is one of the common flowering shrubs in Central India. The plant parts were collected from the botanical garden of Govt. Science College (Autonomous), Jabalpur. The plant parts *i.e.* the flowers, roots, stems and leaves were separately washed with running tap water for 2 hours and dried under shade condition till a constant weight is achieved. The dried plant parts were then grinded in a mixer grinder and the powder to sieve *via* 100 µm mesh. The resultant powder was kept in an air tight container until use.

Extraction of Plant Parts: The dried plant part powders were extracted sequentially with solvents of decreasing polarity. Ten grams of each plant part powder was extracted first using cold percolation technique by suspending the powder in 2L of distilled water for 48 h at 2-8 °C. The suspension was then filtered using Whatman no. 1 filter paper. The filtrate was the aqueous extract. The residue was extracted further with ethanol using Soxhlet extraction method. The residue was subjected further to extractions with ethyl acetate and petroleum sprit (hexane). All the four extracts were dried in vacuo, and the resulting powder was redissolved in 20 ml of their respective solvent.

Total Phenolic Content Estimation: The total phenolic content was determined by the Folin-Ciocalteu method described by Wu *et al.,* (2003) [5]. For this, 1.5 ml of Folin-Ciocalteu reagent (SRL, India) and 1.2 ml of 75% (w/v) sodium carbonate solution was taken in test tubes and 0.3 ml of plant extract from different parts of *N. arbor-tristis* was added to the tubes. The tubes were vortexed for 15 sec and allowed to stand for 30 min at room temperature. Absorbance was measured at 765 nm with the spectrophotometer (EI, India). Results were expressed as milligram of gallic acid equivalent per gram of extract weight.

Peroxide Scavenging Activity: Ferric thiocyanate (FTC) method was used to determine the ability of plant parts extracts to scavenge peroxide radicals, produced during oxidation of lipids (Kikuzaki and Naktani, 1993) [6]. For FTC method, all the extracts of different plant parts of *N. arbor-tristis* were dried completely using a rotary vacuum evaporator to get the powder forms of the extracts. For each extract, 4 mg of this powder was dissolved in 4 ml of ethanol by stirring and filtered into dark bottles using syringe filters (Axiva, India). 4.1 ml of 2.52% of linoleic acid (Sigma, USA) in methanol, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in the same dark vial with a screw cap and then placed in an oven at 40 °C in dark. This served as stock solution. From the stock, each day 0.1 ml of the solution was withdrawn. 9.7 ml of 75% of ethanol, 0.1 ml 30% of ammonium thiocyanate and 0.1 ml of 0.02 M ferrous chloride prepared in 3.5% of hydrochloric acid was added to this solution. Exactly 3 min after the addition of ferrous chloride to the reaction mixture, the absorbance was measured at 500 nm using a spectrophotometer. A negative control with ethanol only and a positive control with ascorbic acid (4 mg ml^{-1}) were placed simultaneously. The process was repeated every 24 h until the absorbance of the positive control became stable.

Calculation:

$$\% \text{ activity} = 100 - (A_1/A_0) \times 100$$

A_0=Absorbance of ethanol reaction.
A_1=Absorbance in the presence of sample extract.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical Scavenging Activity: DPPH free radical scavenging assay using 2, 2, diphenyl-1-picrylhydrazyl (DPPH) stable free radical was done spectrophotometrically using the method of Kumar *et al.,* (2008) [7]. For DPPH stock solution, 2.366 mg of DPPH free radical (Sigma, USA) was dissolved in 100 ml of absolute ethanol to obtain a 60 µM DPPH free radical solution. All the extracts from different plant parts of *N. arbor-tristis* were dried as described in FTC method. 25 mg of dried extract was dissolved in 25 ml of absolute ethanol and kept in dark. Ascorbic acid in 1 mg ml^{-1} concentration served as a positive control. The sample solution of each tested material (500µl) was mixed with the same volume of DPPH solution and allowed to

stand for one and a half hour at room temperature in dark. The absorbance was then measured at 517 nm using a spectrophotometer. Ethanol served as negative control. The percentage scavenging effect was determined by comparing the absorbance of the solution containing the test sample to that of negative control solution (ethanol) without test sample taking corresponding blanks. The absorbance was taken three times each after 30min till the stable readings are achieved. The mean of three measured values for each sample were taken.

Calculation:

% antioxidant activity for DPPH = (A-Ax)/A x 100

Where: A- Absorbance of DPPH solution with ethanol; Ax- Absorbance of DPPH solution with test solution.

RESULTS: The four plant parts of *Nyctanthes arbor-tristis* were extracted sequentially with four solvents of decreasing polarity in order to isolate the different phytochemicals on the basis of their chemical properties. These extracts were dried *in vacuo* and the resulting powder was used for experiments. **Table 1** shows the total phenolic content of the four extracts from different plant parts. The results were expressed as gallic acid equivalent, calculated from the standard calibration curve of gallic acid (y= 0.003X, R^2=0.989), prepared using the same method.

The highest phenolic content was observed with flower, followed by stem, primarily by aqueous and methanolic extracts. Further, ethyl acetate extract of root and stem also showed good amounts of phenolic contents.

TABLE 1: TOTAL PHENOLIC CONTENT IN DIFFERENT PLANT PARTS OF *NYCTANTHES ARBOR-TRISTIS* EXTRACTED WITH DIFFERENT SOLVENTS. THE DATA ARE PRESENTED IN TERMS OF mg EQUIVALENT OF GALLIC ACID AS MEAN ± STANDARD DEVIATION

Plant part	Total Phenolic Content (mg equivalent gallic acid)			
	Aqueous	Methanol	Ethyl Acetate	Hexane
Flower	303.0 ± 23.1	206.3 ± 12.8	12.3 ± 1.1	49.3 ± 2.1
Root	14.0 ± 2.6	9.6 ± 1.7	100.6 ± 2.9	42.0 ± 3.0
Stem	277.0 ± 31.2	84.2 ± 2.9	112.6 ± 4.9	54.0 ± 2.6
Leaf	17.3 ± 2.8	259.6 ± 11.8	84.0 ± 3.1	189.6 ± 3.1

The DPPH free radical scavenging activity of different plant parts extracted with four solvents is presented in **Table 2**. The extracts corresponded to 4 mg ml^{-1} extracted dry weight. The ascorbic acid, in same concentration showed 80.86% activity in comparison to negative control. The methanol and ethyl acetate extracts from all four plant parts showed decent antioxidant activities; with methanolic extract of *N. arbor-tristis* stem showed activity as 86.9%, higher than the positive control. All the ethyl acetate extracts showed moderate to good activity (54.70-65.2%), showing that all the four plant parts have decent antioxidant potential. On the other hand, hexane extracts of leaves showed very good activity *i.e.,* 64.3%.

TABLE 2: ANTIOXIDANT ACTIVITY IN TERMS OF DPPH FREE RADICAL SCAVENGING ABILITY OF DIFFERENT PLANT PARTS OF *NYCTANTHES ARBOR-TRISTIS* EXTRACTED WITH DIFFERENT SOLVENTS. THE PERCENTAGE ACTIVITY OF THE DPPH SCAVENGING ACTIVITY WAS CALCULATED AGAINST BLANK. ASCORBIC ACID (4 mg ml^{-1}) SERVED AS A POSITIVE CONTROL

Plant part	Ascorbic Acid	DPPH Free Radical Scavenging Activity (%)			
	(4 mg ml^{-1})	Aqueous	Methanol	Ethyl Acetate	Hexane
Flower	80.86%	30.0	69.5	55.6	14.7
Root		29.5	60.7	54.7	21.7
Stem		43.4	86.9	54.7	15.6
Leaf		36.5	45.2	65.2	64.3

In order to ascertain the data obtained above, the peroxide free radicals produced during lipid peroxidation was also investigated. **Table 3** shows that in comparison to 92.9% activity of ascorbic acid, the methanolic extracts of flower and root, and ethyl acetate extract of stem showed almost 80% activity. The aqueous extract of leaf showed around 70% of peroxide scavenging activity. Remarkably, the hexane extract of leaf showed the highest (87.0%) peroxide scavenging activity.

TABLE 3: ANTIOXIDANT ACTIVITY IN TERMS OF DELAYING THE LIPID PEROXIDATION ABILITY OF DIFFERENT PLANT PARTS OF *NYCTANTHES ARBOR-TRISTIS* EXTRACTED WITH DIFFERENT SOLVENTS. THE PERCENTAGE ACTIVITY OF THE PEROXIDE SCAVENGING ACTIVITY WAS CALCULATED AGAINST BLANK. ASCORBIC ACID (4 mg ml^{-1}) SERVED AS A POSITIVE CONTROL

Plant part	Ascorbic Acid (4 mg ml^{-1})	Lipid peroxides scavenging activity (%)			
		Aqueous	Methanol	Ethyl Acetate	Hexane
Flower	92.9%	14.8	81.2	46.2	14.8
Root		19.6	79.6	48.1	18.5
Stem		48.1	69.4	79.6	50.2
Leaf		70.3	62.9	70.3	87.0

DISCUSSION: Traditional Indian medicine system has used locally available plants and herbs for all the medical ailments, and this vast knowledge is of importance even today. Antioxidants are documented in several publications to mitigate the inflammatory processes and some of the antioxidant activities of plants have been ascribed to the phenolic compounds [8].

The preliminary phytochemical analysis of extracts indicated the presence of terpenoids, flavonoids, phenols, tannins, alkaloids, glycosides, saponins, fats and fixed oils, proteins and amino acids. The hydro-alcohol and chloroform extracts of whole plant of *N. arbor-trsitis* were found to possess strong *in-vitro* and *in-vivo* antioxidant activity which significantly attenuated paw edema in rats [9]. However, the study did not mention the antioxidant potential, as well as total phenolic compounds in selective parts. The studies using flower of *N. arbor-tristis* deal with methanolic/ethanolic extracts that have shown antioxidant activity in significant dose dependent inhibition of DPPH, 2, 2'- azino- bis(3- ethylbenzothiazoline- 6- sulphonic acid (ABTS) and nitric oxide activity [3, 10].

Recently, Ghosh *et al.*, (2015) were successful in identifying a water soluble polysaccharide with potent antioxidant activity from leaves of *N. arbor-tristis* [11]. Our study also indicates that aqueous extract of leaves have potent antioxidant activity. Since, our results show that aqueous extract of leaves was low in total phenolic content; it can well be assumed that this activity was not due to usual polyphenols but possibly by polysaccharides with esterified phenolic groups. flavonoids from leaves of *N. arbor-tristis* have been shown to posses antioxidant activities [12]. Our study highlights the antioxidant potential of stem of *N. arbor-tristis* apart from the usual plant parts like flower, and to some extent leaves. Being a native plant in most of the India, the easy availability of stem of *N. arbor-tristis* is more suitable for anti-inflammatory activities rather than flowers, which bloom only during the night. The study highlights the antioxidant activities of different plant parts in context to the total phenolic contents, and needs further investigations of the *N. arbor-tristis* stem for possible phytochemicals exhibiting the strong antioxidant activities.

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CONFLICTS OF INTEREST: Nil

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