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Research Article

A comparative study of antioxidant potential in leaves extract of *Nyctanthes Arbor-Tristis Linn*

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ABSTRACT

Nyctanthes arbor-tristis linn (*N. arbor-tristis*) leaves was extensively used in Ayurveda medicine system for the healing of numerous aliments and also found to contain pharmacological and biological activities. The study was aimed to compare the antioxidant potential present in the *N. arbor-tristis* leaves. The fresh leaves of selected plant *N. arbor-tristis* were subjected to preliminary phytochemical qualitative analysis by specific methods. These phytochemical qualitative analysis indicated the existence of Carbohydrates, Terpenoids, Flavonoids, Glycosides, Tannins, Saponins, and absence of Alkaloids and Phlobatannins. Along with these the level of enzymatic antioxidants for example catalase, peroxidase and glutathione-s-transferase, and non-enzymatic antioxidants for example polyphenol, ascorbic acid and total carotenoids are also estimated. The outcome demonstrated a significant level of $P<0.001$ in catalase, polyphenol and total carotenoids when compared to peroxidase, glutathione-s-transferase and ascorbic acid with a significance of $P<0.05$.

Keywords: Ascorbic acid; Catalase; Peroxidase; Glutathione-s-Transferase; Polyphenol.

INTRODUCTION

Free radicals are highly reactive species such as reactive oxygen species (ROS) and Reactive Nitrogen species (RNS) are generated throughout routine cellular metabolism, which are capable of inducing oxidative destruction to biomolecules such as carbohydrates, proteins, lipids and DNA. These liberated free radicals can effect pathological circumstances such as ischemia, anemia, cancer, diabetes, asthma, inflammation, neurodegeneration, arthritis, atherosclerosis, mongolism, parkinson's diseases, ageing process and possibly dermantias (Kris-Etherton *et al.*,2002; Emerit *et al*., 2004; Ara and Nur, 2009). Antioxidants acts as a major defense mechanism against the destruction triggered by free radicals. Numerous plants and plant isolates have been stated to defend the cells from free radical induced damage.

In this respect, our present investigation was designed to display the antioxidant activities of leaf extracts of *Nyctanthes arbor tristis* (NAT).

Nyctanthes arbor tristis (NAT) ordinarily known as Night jasmine (english) or coral jasmine and pavazhamalli (Tamil) belong to the family Oleaceae. It occurs has a large shrub. It is usually scattered in sub-Himalayan region and also cultivated all over India as a garden plant. The leaf extract is broadly used in Ayurveda Medicine for the management of several diseases such as arthritis (Kirtikar and Basu, 1935; Shri Guiabkumaverba Ayurvedic Society, 1949; Gupta *et al*., 2006; Khatune *et al*., 2001), chronic fever, rheumantism, cough (Saxena *et al.,* 2002), skin diseases, ulcer, constipation, and as hair tonic (Saxena *et al.,* 1984), digestives, transquilizer (Saxena *et al.,* 1987) and laxative. This plant leaves has also been found to have pharmacological and biological activities like anti-inflammatory (Guptha *et al*.,1993), analgesic, antipyretic (Badma *et al.,*1988), anti-allergic (Misra *et al*.,1991), anti-malarial (Singh *et al.,* 1992), leishmanicidal (Tandon *et al.,* 1991), amoebicidal (Chitravanshi *et al*., 1992), antihelmintic (Lai *et al.,* 1976), hepatoprotective (Hukkeri Kusum *et al*.,2006), and antioxidant activity (Rathee *et al*.,2007).

In the present study, we attempted to compare the enzymatic antioxidants (catalase, peroxidase and glutathione-s-transferase) and non- enzymatic antioxidants (polyphenols, ascorbic acid and carotenoids) from the fresh leaves of *N. arbor-tristis.*

MATERIALS AND METHODS

Plant material

The fresh leaves of *N. arbor-tristis* were collected first in the morning from D.K.M. College for women,

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Garden, Vellore, TN during the months of Jan/Feb.

Preparation of extract

Collected fresh leaves were rinsed with tap water, washed with distilled water and gently blotted between the folds of filter paper. This sample were used for various assay and estimation. The enzymatic and non-enzymatic antioxidants were assessed as follows.

Estimation of Enzymatic Antioxidants:

Catalase activity

The leaf sample was homogenized in a prechilled mortar and pestle with 0.067M phosphate buffer, P^H 7.0 and centrifuged. The supernatent was used for the assay. The leaf extract ranging from 0.1ml to 0.5ml of the supernatent and 3ml of H_2O_2 phosphate buffer was added together. This was read against a control of phosphate buffer. The control was carried out without addition of H_2O_2. All the samples with absorbance values were measured at 230nm using a spectrophotometer. The enzyme activity was expressed as units per milligram protein (Luck, 1974).

Peroxidase activity

Assay of peroxidase activity was carried out according to the procedure Malik and Singh (1980). Fresh sample was homogenized with 5ml of 0.1M phosphate buffer (p^H6.5) in a homogenizer. The homogenate was centrifuged at 300g for 15mins. The supernatant was used as the enzyme source. To 3ml of 0.05M Pyrogallol solution, 0.1 to 0.5ml of the enzyme extract was added. To this 0.5ml of 1 % H_2O_2 was added. The added mixture was shaken well, and the absorbance value was measured in the spectrophotometer at 400nm. Water acts as a blank reagent was used in the assay. Activity of peroxidase enzyme was expressed in unit time per mg of protein.

Glutathione-s-transferase (GST) Activity

The fresh leaf sample was homogenized with tris-Hcl buffer (p^H7.2). The homogenate was filtered and the filtrate obtained was centrifuged at 4̊C for 30mins at 8,500 rpm. The supernatant was used as the enzyme source. 0.5ml of 0.5M phosphate buffer (p^H6.5), 0.1ml of 25mM CDNB (prepared in 95% ethanol) and 8.8ml of distilled water was added and incubated at 37^0c for 10mins. To the reaction mixture, 0.5ml of 20mM glutathione and 0.2ml of enzyme extract was added. The absorbance of all the samples was read against a control blank. The blank was taken as like the test except the addition of the enzyme. The absorbance was read in spectrophotometer adjusted at 320nm. This was determined by the method (Beutler, 1984). The values for GST activity were expressed in nmol/min/mg.

Estimation of non- enzymatic antioxidant

Polyphenols

Polyphenol assay was followed as according to the method described by (Javanmardi *et al.*, 2003). The samples were homogenized with 80% ethanol. The collected supernatent was saved. The obtained residue was re- extracted with 80% ethanol. The collected supernatent was evaporated to dryness. The residue was dissolved in distilled water. From that 0.1ml to 0.5ml was taken. Then the volume was made upto 2.5ml with water. To this 0.5ml of diluted folincoicalteau reagent and 2.0ml of 7.5% sodium carbonate was added and mixed well. The solution was incubated in a boiling water bath at 45°C for 15mins. The absorbance values were measured using a UV spectrophotometer at 765 nm. The obtained results were measured as mg of Gallic acid equivalent per gm weight.

Ascorbic acid

Ascorbic acid was measured colorimetrically as described by Sadasivam and Manickam (1996). The sample was homogenized in 4% TCA and centrifuged. To the supernatant a pinch of charcoal was added and centrifuged. The supernatant was taken (0.1 to 0.5ml) and make upto 3ml with distill water then added 3ml of DNPH reagent. This is followed by addition of 1-2 drops of thiourea and thoroughly mixed and incubated at 37°c for 3 hrs. The orange- red osazone crystals resulted after incubation were dissolved by adding 7ml of 80% H_2So_4, in cold drop by drop. Incubate it for 30mins at room temperature, the absorbance was read calorimetrically at 540nm. The units were expressed as ascorbic acid equivalents per gm.

Total carotenoids

Assay of total carotenoids was followed according to (Zakaria *et al*., 1979). To the sample 12% alcoholic KOH is added. Then the extract was saponified in a shaking water bath at 37^0c, followed by addition of petroleum ether. The upper petroleum ether layer with the carotenoid pigment is used for the experiment and the lower aqueous phase was removed until it became colourless. In order to remove the turbidity present in the petroleum ether extract, a small quantity of anhydrous Na_2SO_4 was added. The absorbance of the petroleum ether extract was measured at 450nm. Reagent blank serves as control was also used.

Preliminary Phytochemical Qualitative Analysis

Specific qualitative phytochemical tests were performed to know the presence of photo-chemicals (Agarwal 2000; Finar 1975).Viz., alkaloids, Terpenoids, Carbohydrates, tannins (Folin, Coicalteau 1927), Glycosides (Ganjewala *et al*.,2000), Flavonoids (Marinova *et al*., 2005), Saponins and phlobatannins in fresh leaf extract.

Statistical analysis

All data's were expressed as means ± S.D. Two way analysis of variance (ANOVA) and Bonferroni multiple comparision test were carried out using SPSS-16 pack-

Table 1: Preliminary phytochemical qualitative analysis of *N. arbor tristis*

Phytochemicals	Result
Alkaloids	-
Terpenoids	+
Carbohydrates	+
Glycosides	+
Saponins	+
Tannins	+
Flavanoids	+
Phlobatannins	-

(-) =Absence, (+) = Presence

Table 2: Enzymatic Antioxidant activity of the Leaves of N.arbor Tristis (NAT)

Parameters	Fresh leaves (NAT)				
	0.1ml	**0.2ml**	**0.3ml**	**0.4ml**	**0.5ml**
Catalase	1.151±0.02**	1.220±0.03*	1.235±0.02**	1.256±0.03**	1.270±0.02**
Peroxidase	0.719±0.001*	0.829±0.002*	0.835±0.001*	0.842±0.002*	0.855±0.01*
Glutathione s-transferase	1.096±0.01**	1.107±0.02**	1.110±0.04**	1.148±0.02**	1.191±0.04**

Values are expressed as Mean±SEM of three enzymes.
Significance P*=0.05, P**=0.001
Unit of catalase (unit/mg of protein)
Unit of Peroxidase (unit time / mg of protein)
Unit of Glutathione-s-transferase (nmol/min/mg).

Table 3: Non- Enzymatic antioxidant activity of the leaves of N.arbor tristis (NAT)

Parameters	Fresh leaves (NAT)				
	0.1ml	**0.2ml**	**0.3ml**	**0.4ml**	**0.5ml**
Polyphenol	1.630±0.02**	1.654±0.05**	1.736±0.04**	1.821±0.02**	1.950±0.03**
Ascorbic Acid	0.921±0.001*	1.076±0.03**	1.082±0.01*	1.086±0.04*	1.109±0.001*
Carotenoids	1.062±0.03**	1.076±0.02**	1.089±0.03*	1.099±0.01*	1.180±0.01**

Values are represented as Mean±SEM of three enzymes
Significance P*=0.05, P**=0.001

age to determine the significant differences with p values of <0.05 and <0.001.

RESULTS AND DISCUSSION

In recent years, the documentation and examination on plant antioxidants from medicinal plants is acting as emergent field in research and numerous antioxidants have been studied. The recent study is to show antioxidant property in leaves of *N. arbor-tristis.* Table 1 show the presence of secondary metabolites such as Carbohydrates, Flavonoids, Terpenoids, Glycosides, Saponins, and Tannins and absence of alkaloids, phlobatannins in fresh leaves extract were confirmed after performing specific qualitative tests. (Priya and Deepak Ganjewala 2007; Narendhirakannan Ramasamy Thangavalu and Smeera Thomas 2010; Kusum *et al*., 2009).

All these phytochemical analysis of extract of the leaves of *N. arbor-tristis* revealed the presence and lack of some of the phytochemical compounds. These phyto compounds have been known as antioxidant agents, known to act as free radical terminators. (Trease and Evans 1983; Shahidi and Wanasundara 1992; Sofowora 1993; Nanu Rathod *et al.*, 2010)

In the present report, among the selected three enzymatic antioxidants catalase, peroxidase and glutathione-s-transferase, catalase exhibited a maximum (P<0.001) activity (Nanu Rathod *et al.*, 2010) in the *N. arbor-tristis* leaves, than the activity of peroxidase and glutathione-s-transferase (P<0.05). (Table 2)

Catalase, peroxidase and glutathione-s-transferase are endogenous enzyme systems that play a vital activity in the cells. These enzymes are responsible for the removal of H_2O_2 as well as other organic hydroperoxides thereby protecting the cell against the oxidative damage (Gessier *et al*., 2007; Ramapriya and Usha, 2010).

It is evident from Table 3 that the non-enzymatic antioxidants Polyphenol (Duch *et al.,* 1999; Gordon, 1990) and carotenoids were comparatively significant P<0.001 than ascorbic acid with a significance of P<0.05 (Ramapriya and Usha, 2010).

Polyphenols, ascorbic acid, carotenoids catalysis the conversion of H_2O_2 which damages the cell. Thus these three non-enzymatic antioxidants also have a major role in antioxidant property. (Liszkay, 2005) reported that enzymatic and non-enzymatic antioxidants presence in the photosynthetic organism like leaves, can quench the reactive singlet oxygen produced during photosynthesis via triplet chlorophyll formation.

Our result, showed a higher activity of catalase, polyphenol and carotenoids in the leaves of *N. arbor-tristis* when compared to other cases like peroxidase, glutathione-s-transferase and ascorbic acid. These higher activity are due to the presence of chlorophyll in leaves of *N. arbor-tristis.* Phytochemical analysis reveals the occurrence of phytochemicals which also have antioxidant property.

Based on the present results, it was observed that *N. arbor-tristis* leaves extracts has free radical scavenging activity and improved high antioxidant effect. These findings suggest that the *N. arbor-tristis* leaf extract can be used to cure the diseases caused by oxidative stress.

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