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Antioxidant and Anti-inflammatory Activities Of *Nyctanthes Arbor-Tristis* Extracts

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ABSTRACT

Oxidative stress plays an important role in chronic complication of diabetes and is postulated to be associated with increased lipid peroxidation. Novel type of antioxidants was identified from *Nycanthes arbor-tristi*. The antioxidant activity was analyzed for the ethanol extracts of *Nyctanthes arbor-Tristis* [leaf,flower and stem] by using the DPPH method, total phenol content, Hydrogen peroxide assay, Reducing power assay, and lipid per oxidation assays. It is observed that *Nyctanthes arbor-Tristis* act as the source of natural antioxidants. Significant anti-inflammatory activity was observed on *Nyctanthes arbor-Tristis* [leaf,stem,and flower] extracts by using HRBC method.

Key words: Nyctanthes arbor-Tristis antioxidant activity, anti-inflammatory activity.

INTRODUCTION

Natural product is a source for bioactive compounds and has potential for developing some novel therapeutic agent. Over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control.The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment [1]. Nyctanthes arbor-tristis Linn belongs to family Nyctantheaceae. Commonly known as Harishringi. The vernacular names of the plant Nyctanthes arbor-tristis are in Hindi- Parijata, Sanskrit-arsinghar, tamil-Prajaktha, Marathi– Sephalika, Telugu- Night Jasmine [2].

Traditionally used to treat sciatica, arthritis and malaria, however, reports from the literature have also indicated that the leaf oil from *N. arbor-tristis* include hepatoprotective, anti-leishmanial, antiviral and antifungal activities [3]. Other research into the leaf extract of the *N. arbor-tristis* have shown considerable immunological activity and water soluble ethanol extracts from the leaves are reported to possess anti-inflammatory activity which, however, accompany development of ulcers in test rats [4]. The methanol dried fruit extract of *Nyctanthes arbor-tristis* plant was found to be a great source of active metabolites for potent inhibition of tumor cell lines. It showed antiproliferative action against MDA MB-231 cancer cells at 15mg/ml conc [5]. The antioxidant capacity and the amount of polyphenols, flavonoids, and flavonols are present in *Nyctanthes*. Total polyphenols plays a vital role in anti-oxidization as well as in the biological functions of the plant. For example, fruits such as blueberry, cranberry and *Sambucus nigra are* rich in flavonoids that protect endothelial cells from oxidation, a key factor in the development of cardio-vascular diseases [6].

The methanol extracts of dry flower *Nyctanthes* exhibited high antioxidant activities with high total phenol content. Phenol compounds such as flavonoids, phenolic acids possess diverse biological properties such as anti-inflammatory, anticarcinogenic and anti-atherosclerotic activities [7]

Nyctanthes leaf extract ameliorates silica induced early fibrogenic reaction in lungs of mice by depletion of tumour necrosis factor α (TNFα). Free radicals are known to be the source of major causes of various chronic and degenerative diseases including ageing, coronary heart disease, stroke, diabetes mellitus, cancer and inflammation [8]. Previous reports suggested that bark of *Nyctanthes arbor tristis* contains glycosides and alkaloids. The iridoid and phenylpropanoid type glycosides present in the *Nyctanthes* plant bark. Stem of *Nyctanthes arbortristis* contains glycoside-naringenin-4'-0-β-glucapyranosyl-α- xylopyranoside and β-sitosterol [9]. Quorum sensing (QS) is a widespread prokaryotic intercellular communication system based on the signal molecules (autoinducers) relative to cell density. Compounds that interfere with the QSsystem to attenuate bacterial pathogenicity are termed as anti-QS compounds. Inhibition of QS is therefore was considered as the new target for antimicrobial chemotherapy with the current quest on discovering non-toxic QS inhibitors from natural sources [10].

Medicinal plants stimulate the immune system viz *Panax ginseng, Ocimum sanctum, Tinospora cordifolia*, and *Terminalia arjuna*. The presence of secondary metabolites like alkaloids, glycosides, saponins, flavanoids, coumarins, and sterols exhibited as a wide range of immunomodulatory substances [11]. *N arbor-tristis* collected from Jabalpur contains bioactive plant metabolites like alkaloids, tannins and cardicac glycosides act as natural antioxidants. They induce

free radical scavenging, singlet oxygen quenching,chelating of transitional metal such as iron, as well as a reducing agents and activator of antioxidative defense enzyme systems to suppress radical damage in biological system [12]. The objective of this study is to analyze the chemical compositions, and examine the antioxidant and anti-inflammatory activities of *N. arbor-tristis*.

MATERIALS AND METHODS

Collection of plant

The plant *Nyctanthes arbor-tristis* was collected from Ayya Nadar Janaki Ammal college campus, Sivakasi, Virudhunagar district, Tamilnadu. The plant *Nyctanthes arbor-tristis* (NAT) were washed with water. The Arial parts (leaf, stem and flower) of the plant was shade dried at 30 ^{0}C to 32 ^{0}C for 3 days. The dried plants were powdered using motor and pestle and stored in air tight zip lock pouches till extraction.

Preparation of extracts

1 g of powdered samples of plant *Nyctanthes arbor-tristis* was soaked in 10ml of 70 %ethanol for 5 days. The resultant crude extract was centrifuged at 3000 rpm for 20 minutes and supernatant was collected and evaporated at room temperature (~ 32 ^{0}C). The resultant mass was made up to 5 ml using 25% DMSO and used as stock solution (having final concentration of1 mg/5µl) and stored at 0 ^{0}C.

Antioxidant assays

DPPH radical scavenging assay

Free radical scavenging ability of the plant extracts was tested by DPPH radical scavenging assay. Different concentration of sample 5 µl,10µl,20µl,40µl,80µl of the extracts were taken in the test tubes. 3 ml of 0.1mM DPPH in ethanol was added to each tube and incubated in dark at room temperature for 30 min. The absorbance was read at 517nm using UV visible spectrophotometer. Ascorbic acid was used as the standard. The percentage of inhibition was calculated using the formula

% = [Abs (control)-Abs(sample)] x 100/Abs(control)

Hydrogenperoxide (H_2O_2) scavenging assay

H_2O_2scavening activity was determined [13]. A solution of H_2O_2 (10 mM) was prepared in phosphate buffer (PH 7.4). Reaction mixture containing 2.5 ml of H_2O_2 solution and 0.1ml, 0.3ml,0.5ml,0.7ml,0.9ml was made up of to 3ml with phosphate buffer. The absorbance was measured at 240 nm. Ascorbic acid was used as the standard. Total H_2O_2 scavening activity was expressed in %.

HRSA (%) = [Abs (control)- Abs (sample)/Abs (control)] x100

Total phenolic content

Total phenol content was determined with Folinciocalteau reagent according to the method described by [14] using gallic acid as standard. The concentration range 25µl, 50µl,75µl,100µl,125µl of the sample was mixed with 9 ml of distilled water and 1 ml of folin - ciocalteu reagent and 10 ml of 7% sodium carbonate was added. After 90 min of incubation the absorbance was determined at 765 nm. The phenolic content was expressed as GAC in mg/ml.

Reducing power:

The reducing power was estimated according to the method [15] Different concentration of extracts (5µl, 10µl, 15µl, 20µl), along with 0.8 ml of ethanol, 2.5 ml of 200 m mol/L sodium phosphate buffer (pH 6.6), and 2.5 ml of 10 mg/ml potassium ferricyanide, were fully mixed and incubated at 50°C for 20 min. After 2.5 ml of 100 mg/ml trichloroacetic acid was added, 1 ml of reaction mixture solution was mixed with 2.5 ml of deionized water and 2.5 ml of 1 mg/ml ferric chloride. After incubating for 10 min at room temperature, the absorbance of resultant solution was measured at 700 nm. The reaction mixture with 0.2 ml of ethanol for replacing the sample solution was used as the control. A higher absorbance indicated a higher reducing power.

Lipid peroxidation

Lipid peroxidation (LPX) was measured according to the malondialdehyde (MDA) content, a product of LPX estimated by the thiobarbituric acid (TBA) reaction. The different concentration of NAT extract at (0.1, 0.2, 0.3mg) was dissolved in 5 ml of 10% (W/V) trichloro acetic acid (TCA), and centrifuged at 7000 × *g* for 10 min. One ml of the supernatant was mixed with 2mLof 0.5% TBA solution (in 10% TCA). Then the mixture was heated at 95°C for 45 min and then cooled at room temperature. The supernatant was read at 532 nm after the removal of interfering substances by centrifuging at 4000 x g for 10 min.

Anti-Inflammatory assay :

The anti-inflammatory activity of NAT was assessed by *in vitro* HRBC membrane stabilization method.. Fresh blood was collected from healthy voluntaries and mixed with equal volume of sterilized Alsever solution and stored at 4^{o}C and used within 5 hrs. Alsever solution containing 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride. Saline at two different concentrations were prepared (isosaline 0.85% and hyposaline 0.25%). 0.5 M phosphate buffer p^H 7.4 .Add 19 ml 0f 0.5 M sodium dihydrogen phosphate solution to 81 ml of 0.15 M sodium hydrogen phosphate solution. Check the pH and adjust with monobasic or dibasic solutions as required. Store at room temperature for 4 weeks. RBC suspension: The blood samples were centrifuged at 3000 rpm at room temperature for 10 minutes and the packed cells obtained were washed with isosaline (pH 7.2) 3 times and 10% (v/v) suspension was made with isosaline. The assay mixture contained different concentration of NAT extract (200µl, 300µl,400µl,500µl), 1 ml of phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline and 0.5 ml of 10% RBC suspension. In another tube instead of 2 ml of distilled water was taken and this served as the control. All the tubes were incubated at 37^{o}C for 30 min. Then it was centrifuged and the haemoglobin content in the supernatant was estimated using UV-spectrophotometer at 560 nm. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560nm. The percentage of haemolysis was calculated by using the following formula,

Percentage of haemolysis = OD of Test X 100 / OD of Control

The percentage of HRBC membrane stabilization or protection was calculated by using the following formula,

Percentage protection = 100 - OD of Test X 100 /OD of Control

RESULTS AND DISCUSSION

Figure 1 shows the antioxidant activity of plant crude extract of *Nyctanthes arbor tristis* leaf, stem and flower using DPPH method. The absorbance was measured OD at 570nm. It also shows the comparative analysis of the plant crude extracts such as *Nyctanthes arbor tristis* leaf, stem, and flower using DPPH method.Maximum activity was seen in leaf as blue colour. Figure 2 represents the antioxidant activity of *Nyctanthes arbor tristis* leaf, stem and flower extracts using H_2O_2 method. The absorbance was measured OD at 240 nm. It also shows the comparative analysis of the plant crude extracts such as *Nyctanthes arbor tristis* leaf, stem, and flower using H_2O_2 method. Figure 3 suggest the antioxidant activity of *Nyctanthes arbor tristis* leaf, stem and flower extracts using TPC method. The absorbance was measured OD at 240nm. The comparative analysis of the plant crude extracts such as *Nyctanthes arbor tristis* leaf, stem, and flower using TPC method is seen in figure 3.

Figure 4 states the antioxidant activity of *Nyctanthes arbor tristis* leaf, stem and flower extracts using reducing power assay. The absorbance was measured OD at 700nm. It also denotes the comparative analysis of the crude extracts such as *Nyctanthes arbor tristis* leaf, stem, and flower extracts using reducing power assay.Figure 5 reviews that antioxidants activity of *Nyctanthes arbor tristis* leaf, stem and flower extracts using lipid per oxidation assay. The absorbance was measured OD at 542 nm. Figure 5 states the comparative analysis of the crude extracts such as *Nyctanthes arbor tristis* leaf, stem, and flower using lipid per oxidation assay. Figure 6 indicates the anti-inflammatory activity of *Nyctanthes arbor tristis* extracts was measured using HRBC method. Maximum activity was seen in leaf extracts. Table 1 denotes the anti-inflammatory action of NAT extracts measured at 595nm.

Free radicals are generated as part of the body's normal metabolic process. Antioxidants are radical scavengers which shield the human body against the free radical damages. It includes ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Leaf extract of *Artimisia dubia* and *Nyctanthus arbortrtis*, stem extract of *Swertia chirata* and extract from *Ocimum sanctum* efficiently scavenged the DPPH free radical [16]. The 50% ethanolic extract of leaves of *Nyctanthes arbortristis* in experiment showed the significant decrease in the blood glucose level, increase the antioxidant efficacy in alloxan induced diabetes [17). Various parts of the *Nyctanthes arbor-tristis* are used in the treatment of itches, cuts, ulcers, swellings, bilicious fever, catarrh, eczema, dysentery and chest complaints of children, fistula, pustules and rheumatism [18]. The Methanol extracts of *Iris nigricans* (rhizomes, leaves and flowers) were evaluated for their free radical scavenging activity using the DPPH radical assay. The rhizomes extract of *Iris nigricans* were resulted the free radical scavenging of DPPH activity showed moderate activity when compared with that *Iris nigricans* leaf and flower extracts. Because *Iris nigricans* flower and leaf contains the phenolic compounds, flavonoids and saponins. These compounds were capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity [19]. It is observed that *Nyctanthes arbor tristis* ethanol extracts showed the DPPH free radical scavenging activity. From this it is identified that *Nyctanthes arbor tristis* extract of leaf and stem have maximum activity,when compared with that of flower extracts. The leaf and stem have compounds like phenol and flavonoids. The reducing potential can reflect some aspects of antioxidant activity in the extracts of *Euphorbia serrata*. The intensity of colour resulting from reduction of ferric ions depends on the reducing potential of the compounds presented in the extract of flavonoids and alkaloids The intensity of the colour reflects the absorption which is parallel to antioxidant activity, the absorption range is 700nm. He reported the *Euphorbia serrata* leaf extract have a maximum amount of flavonoids, these compounds were used the treatment of anticancer activity also [20]. It is clearly evident that *Nyctanthes arbor tristis* showed reducing potential antioxidant properties in the intensity of ferric ions using the ethanolic extract. This could be due to the presence of flavonoids compounds. It is also found that stem flower and leaf have free radicals which could be agents responsible for antioxidant properties.

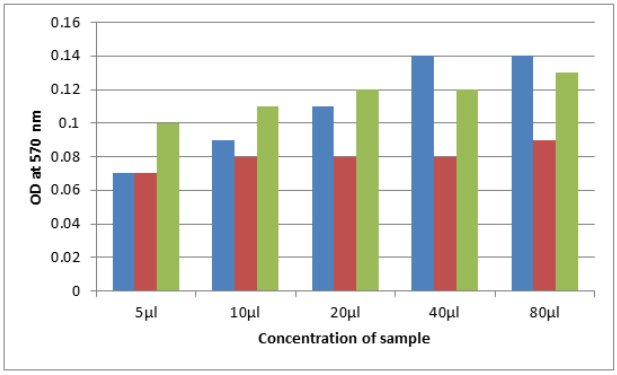

Fig1 : Comparative analysis of antioxidant activity of *Nyctanthes arbor tristis* leaf, flower and stem using DPPH method.

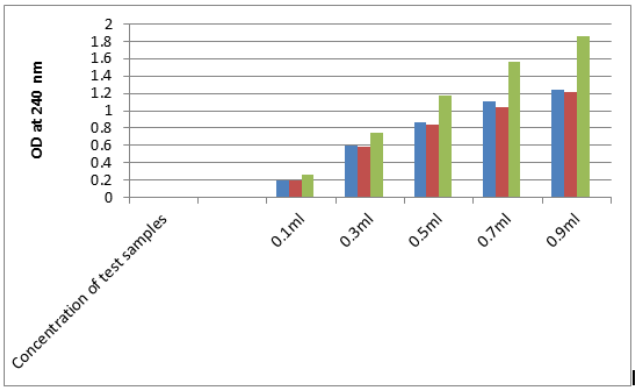

Figure 2: Comparison of antioxidant activity of *Nyctanthes arbor tristis* leaf, flower and stem extracts using H_2O_2 assay

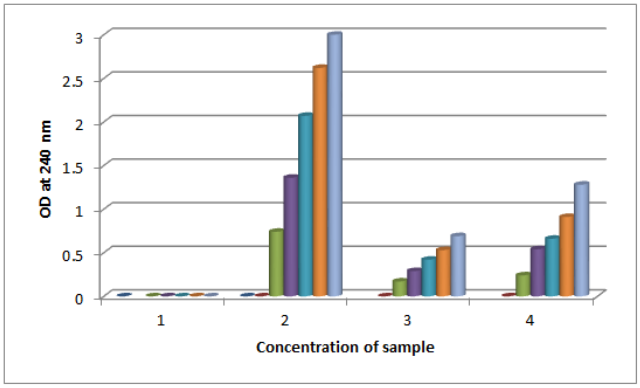

Figure 3: Comparison of antioxidant activity of *Nyctanthes arbor tristis* leaf , flower and stem using TPC method

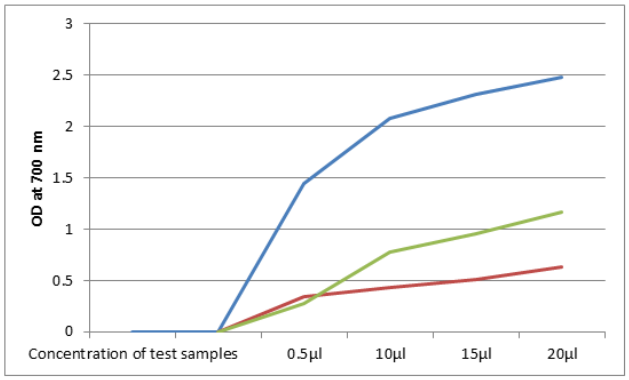

Figure 4: Comparison of antioxidant activity of *Nyctanthes arbor tristis* leaf, flower and stem using reducing power assay

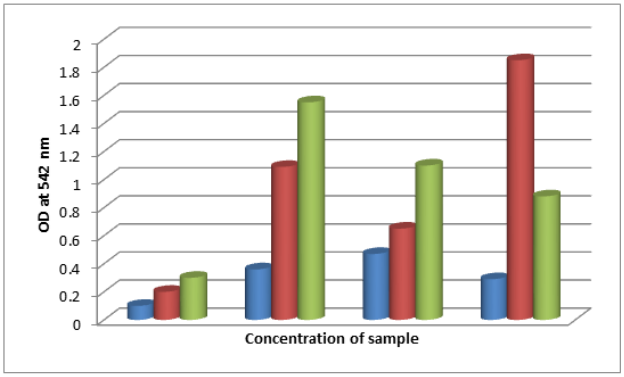

Figure 5: Comparison of antioxidant activity of *Nyctanthes arbor tristis* leaf, flower and stem using lipid per oxidant

The plant *unica granatum*, showed hydrogen peroxide scavenging and powerful total antioxidant activities when compared to standards such as BHA and α-tocopherol , *unica granatum* extract have bioactive metabolites such as, flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins [21]. Similarly the *Nyctanthes arbor tristis* extract of leaf and flower have flavonoids reduce the phenolic content and free radicals of hydrogen peroxide. The free radicals of less activity in stem extract of *Nyctanthes* and compare then extract of *Nyctanthes arbor tristis* leaf and flower.The inhibition of hypotonicity induced HRBC membrane lysis i.e, stabilisation of HRBC membrane was taken as a measure of the anti-inflammatory activity. The percentage of membrane stabilisation for methanolic extracts and Diclofenac sodium were done at 50, 100, 250, 500, 1000, 2000 µg/ml. The Methanolic extracts of *Centella asiatica* are effective in inhibiting the heat induced hemolysis of HRBC at different concentrations (50-2000µg/ml) . It showed the maximum inhibition 94.97% at 2000µg/ml. With the increasing concentration the membrane hemolysis is decreased and membrane stabilisation / protection is increased [22]. In the present study the *Nyctanthes arbor tristis* leaf extract have the potential activity of anti-inflammatory showed the maximum inhibition range at 1.17% in 500µl concentration.

Table 1: Antiinflamatory activity of HRBC by using *Nyctanthes arbor tristis*

Concentration of test samples	*Nyctanthes arbor tristis* (OD at 595nm)		
	leaf	flower	stem
200µl	3.00	3.00	3.00
300µl	3.00	2.74	3.00
400µl	2.79	2.67	3.00
500µl	1.17	3.00	3.00

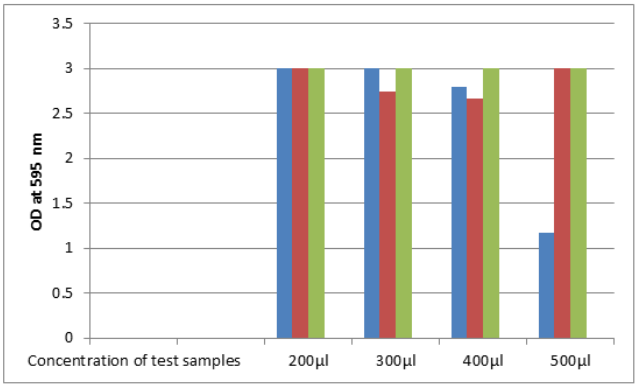

Figure 6: Anti inflamatory activity of HRBC by using *Nyctanthes arbor tristis* extracts.

CONCLUSION

In the present study, we made an attempt to study the antioxidant activity and anti-inflammatory activities for the ethanol extracts of *Nyctanthes arbor-Tristis* Based on the results obtained, we can conclude that the plant *Nycanthes arbor-Tristis* could be used for the development of pharmaceutical drugs.

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