Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 11, 2014

Original Article

COMPARATIVE ANALYSIS OF THE *IN VITRO* ANTIOXIDANT ACTIVITY AND POLYPHENOLIC CONTENT OF SUCCESSIVE EXTRACTS OF *NYCTANTHES ARBOR-TRISTIS* LINN.

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Received: 06 Oct 2014 Revised and Accepted: 04 Nov 2014

ABSTRACT

Objective: Present work comprehensively evaluates the antioxidant capacity, total phenolic content (TPC) and total flavonoid content (TFC) of four successive extracts of various parts of *Nyctanthes arbor-tristis* Linn. at different concentrations using spectrophotometric assays.

Methods: TPC and TFC were measured using Folin–Ciocalteu's and aluminium trichloride method, respectively. Antioxidant activity was evaluated by Trolox-equivalent antioxidant capacity and 2, 2-diphenyl-2-picrylhydrazyl (DPPH) assays. Relationship between free radical scavenging assays and phenolic compounds was deduced using correlation matrix between various study parameters. TPC, TFC and antioxidant activity was higher in methanolic extracts followed by aqueous, petroleum benzene and chloroform extracts.

Results: Methanolic and aqueous extracts were found to have higher phenolic and flavonoid content. Antioxidant capacity of methanolic and aqueous extracts were greater than petroleum ether and chloroform extracts.

Conclusion: The study suggests that to a certain extent, all extracts act as radical scavengers possibly due to presence of polyphenolic compounds. It was concluded that *Nyctanthes arbor-tristis* Linn. exhibit strong antioxidant activity and could serve as potential therapeutic plant for various diseases.

Keywords: Antioxidant activity, Phenols, Flavonoids, Plant extracts, Correlation.

INTRODUCTION

Plants had been used by mankind since antiquity as food, medicines and industrial raw material. Phytometabolite studies have attracted interest of R&D over many years. These compounds play an important role in the adaptation of plants to their environment and also, represent a major source of active pharmaceuticals[1]. Imbalance in pro-oxidants and antioxidants causes oxidative stress in the biological system [2].

Oxidative damage is a crucial event in a complex interplay of different mechanisms in both normal aging and degenerative diseases[3]. Antioxidants, potentially delay or prevent oxidation of the substrate, even at low concentrations[4]. Axiomatically, antioxidants perhaps play a considerable role in preventing the onset and/or the progression of oxidative pathologies and provide protection to foods[5].

Plants are recognized as great source of natural antioxidants by the traditional medicinal systems. Plant polyphenolics such as flavonoids, tannins, etc. possess free radical-scavenging properties because of their favorable structural chemistry[6]. Several studies have recommended consumption of food items rich in antioxidant to augment array of free radical scavengers inside the body.

For past many years nutraceutical antioxidants have attained extensive attention of researchers on the grounds that they are likely to be promising, safe and effective with least side effects. Therefore, they serve as substantial source for the development of novel drugs to prevent and/or cure chronic human diseases [7]. Also, natural antioxidants may serve as alternative to prevent deterioration of stored food products.

Many protocols either using commercially obtainable radicals (DPPH, ABTS, ORAC, TEAC, etc.) or metal ions (like FRAP, LDL, etc.) to determine the total antioxidant activity are available [8]. It is recommended to base any conclusions on at least two different test systems, because various aspects such as test system used, solvent system etc. affect the measurement of the antioxidant activity measured [9]. Therefore, two assays namely, DPPH and TEAC inhibition assays were employed for the present study.

Nyctanthes arbor-tristis Linn. commonly known as Night Jasmine or Parijata, is a large shrub belonging to family Oleaceae. It is native to India, distributed in Bangladesh, Indo-Pak subcontinent and South-East Asia, tropical and sub-tropical South East Asia. *Nyctanthes arbor-tristis* is a medicinally valuable, sacred and ornamental shrub known across the country for its fragrant white flowers. Almost all the parts of this plant: root, bark, leaves, flowers, seeds and seed oil are medicinally as well as industrially important plant[10]. Different parts of this plant are used in Indian systems of medicine for various pharmacological actions such as antiasthmatic, hepato protective, anticancerous, hypoglycemic effects, antileishmaniasis, antiviral, antifungal, antibacterial, antipyretic, antihistaminic, antimalarial, anti-inflammatory, antioxidant activities [11-12]. It is considered as one of the five wish-granting trees of Devaloka in Hindu mythology thence, flowers garlands are made for religious offerings. Orange dye obtained from flowers is used by textile industries for dyeing cotton and silk [13].

To the best of our knowledge, there are only a few detailed data available on free radical scavenging and/or antioxidant properties of *N. arbor-tristis.* The present study comprehensively investigates antioxidant capacity, total phenolic and flavonoid content of successive extracts of various parts of *N. arbor-tristis* at different concentrations using spectrophotometric assays. Also, to find out relationship between free radical scavenging assays and phenolic compounds correlation between various study parameters was determined.

MATERIALS AND METHODS

Plant samples

Different parts, namely, flowers (NF), seeds (NS), leaves (NL), roots (NR) and bark (NB) of *N. arbor-tristis* (Voucher No. - RUBL 21188) were collected from Jaipur, Rajasthan, India during full bloom and were authenticated from Herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.

Processing of plant samples

All collected plant parts were rinsed thrice using distilled water. Then plant samples were weighed and shade dried in a room with

active ventilation and ambient temperature for 10 days. Dried samples were weighed and powdered using a grinder. Finely grinded samples were stored at -20°C in dark air-tight containers for further use.

Chemicals

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), aluminum chloride hydrate, Folin & Ciocalteu's phenol reagent (FCPR), gallic acid, potassium acetate, potassium persulfate, quercetin, sodium carbonate, petroleum benzene, chloroform, methanol were purchased from Sigma-Aldrich, India.

Preparation of the extracts

Powdered samples (50g) packed in a Whatman® cellulose extraction thimbles (Sigma-Aldrich) to prepare successive extracts using different solvents namely, petroleum benzene (PE), chloroform (C), methanol (M) and water (AQ) via a Soxhlet apparatus (Borosil). The extracts so obtained were dried with the help of vacuum desiccator (Tarsons) to get a solid residue. The solid residue was stored in dark colored glass bottles at -20 °C till further use.

Assay for total phenolics (TPC)

The total phenolic content in plant extracts was determined by Folin Ciocalteu's method [14] with slight modifications. One mL of plant extract (10 g/L) was mixed with 3 mL of 10% FCPR (w/v) and 2 mL of 20% sodium carbonate (w/v). This mixture was incubated for 30 minutes on water bath (40 °C) then cooled to room temperature and absorption was read at 765 nm. Gallic acid was taken as standard and calibration curve was prepared using its various aliquots. TPC was calculated by the following formula:

$$P = c * V/e$$

where; P—total content of phenolic compounds, mg/g plant extract, in gallic acid equivalents (GAE); c—the concentration of gallic acid established from the calibration curve, mg/mL; V— the volume of extract, mL; e—the weight of pure plant extract, g.

Assay for total flavonoids (TFC)

The total flavonoid content was determined by pharmacopeia method [15] with minor modifications. One mL of plant extract (10 g/L) was mixed with 1 mL of 10% aluminium trichloride (w/v) and 1 mL of 1M potassium acetate, then volume was raised up to 10 mL with the corresponding solvent. This mixture was then shaken vigorously and incubated for 30 minutes at room temperature. The absorption was read at 415 nm. Quercetin was used as positive control. The absorption of quercetin solutions was measured under the same conditions to plot calibration curve.

$$F = a * V/e$$

where; F — total content of flavonoid compounds, mg/g plant extract, in Quercetin equivalent (QE); a — the concentration of gallic acid established from the calibration curve, mg/mL; V— the volume of extract, mL; e—the weight of pure plant extract, g.

DPPH radical scavenging assay

The antioxidant activity [16] of the plant extracts so prepared was measured spectrophotometrically using stable DPPH. One mL of different concentrations of the extracts was added to a 3 mL of a 0.004% DPPH solution (w/v). After shaking vigorously, the mixture was allowed to stand undisturbed for 30 minutes in complete dark.

The absorbance of the resulting solution was measured at 517 nm with a UV/visible light spectrophotometer (Shimadzu UV 1700, Kyoto, Japan). Blank sample was prepared following same steps but test sample was replaced with same amount of corresponding solvent with each set of experiment. Inhibition activity was calculated in following way:

$$\% \ Inhibition = [(A_B - A_S) / A_B] \times 100$$

where, A_B - absorption of blank sample; A_S - absorption of tested extract solution.

Trolox equivalent antioxidant capacity (TEAC) assay

ABTS radical cation decolorization test [17] is another spectrophotometric method widely used for the assessment of antioxidant activity. Four mL of 2 mM ABTS cation solution were mixed with 100 µL test extract and the decrease of absorption was measured spectrophotometrically at 734 nm during 6 min. The degree of decolorization as percentage inhibition of the $ABTS^{+\cdot}$ radical cation is determined as a function of concentration and time and calculated relative to the reactivity of Trolox as a standard, under the same conditions. Therefore, this assay is often referred to as the Trolox equivalent antioxidant capacity (TEAC) assay.

Statistical analysis

Analyses were run in triplicates and the results were expressed as mean values with standard error mean. Correlation coefficients (R) to determine the relationship among different antioxidant assays, TPC and TFC were calculated using MS Excel software (CORREL statistical function).

RESULTS AND DISCUSSION

Extraction method and solvent selection

Soxhlet apparatus was used in the present study to derive sequential extraction of the plant material to clinch maximum compounds, even those with limited solubility in a solvent. In order of increasing polarity, four different solvents (PE, C, M and AQ) were employed for this purpose. Benefits of this method is that comparative data for different extraction solvents is obtained that abridges identification of the active compounds in the crude extracts so obtained via their further fractionation.

Content of phenolic and flavonoid compounds

Plants produces vast variety of primary and secondary metabolites. Phenolic compounds are one of the most important groups of secondary metabolites. Structural chemistry of phenolics is quintessential for free radical scavenging as it possesses at least one aromatic ring (C6) bearing one or more hydroxyl groups. In general, phenolic compounds act as potential metal chelators as well as inhibit lipid per-oxidation by quenching free radicals via forming resonance-stabilized phenoxyl radicals. Among the multifarious phenolic compounds, flavonoids are probably the most important class. They are be endowed with ability to directly scavenge reactive oxygen speciesby readily donating electrons or hydrogen atoms [18]. Therefore, TPC and TFC were investigated (Fig. 1 and 2).

The TPC (mg/g), determined from regression equation of calibration curve ($y = 0.0018x - 0.0469$, $R^2 = 0.99$) and expressed in GAE, varied between 4.34 and 94.78. Recovery of phenolic content in the extracts was in accordance with previous studies which states highest recovery in M extracts followed by AQ, PE and C extracts[19]. Highest value of phenolic content was observed in M extract of NL (94.78 mg/g in GAE) whereas PE extract of NR showed lowest value (7.44 mg/g in GAE). M extract of NS possessed high TPC (62.79 mg/g in GAE). M and AQ extracts were observed to be richer in TPC than PE and C extracts. This suggested more presence of polar compounds than non-polar compounds.

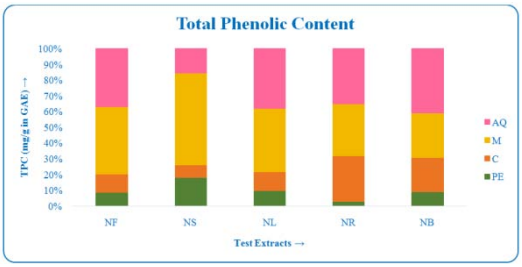

Fig. 1: Total phenolic content of different test extracts. {Petroleum ether (PE), chloroform (C), methanol (M), water (AQ) extracts; *Nyctanthes arbor-tristis* flowers (NF), seeds (NS), leaves (NL), roots (NR) and bark (NB); Gallic acid equivalent (GAE), Total phenolic content (TPC)}.

The TFC (mg/g), determined from regression equation of calibration curve (y = 0.6942x - 0.0042, R^2 = 0.99) and expressed in QE, varied between 2.97 and 65.64. M and AQ extracts showed significantly higher flavonoid content followed by C and PE extracts. Highest concentration of flavonoids was observed in M extract of NL whereas lowest was recorded in PE extract of NR. M extracts of NS, NF also exhibited high TFC (93.33 and 92.77 mg/g in QE respectively) which suggests presence of polar compounds in *N. arbor-tristis.*

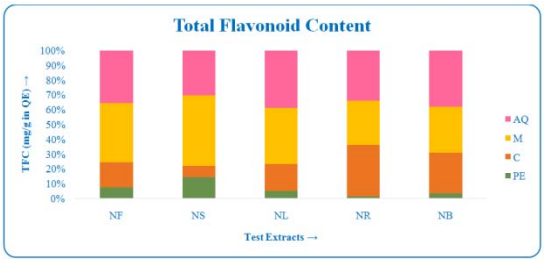

Fig. 2: Total flavonoid content of different test extracts. {Petroleum ether (PE), chloroform (C), methanol (M), water (AQ) extracts; *Nyctanthes arbor-tristis* flowers (NF), seeds (NS), leaves (NL), roots (NR) and bark (NB); Quercetin equivalent (QE), Total flavonoid content (TFC)}.

Antioxidant activity

Fig. 3 reveals results of TEAC assay and fig. 4,5,6,7 and 8 displays results of DPPH assay of different plant parts. Both the assays are based on transfer of electron leading to reduction of the colored oxidant. The concentration of the antioxidants present in a sample can be determined spectrophotometrically as the degree of the color change.

TEAC is appropriate to both hydrophilic and lipophilic antioxidants. In the other hand, the DPPH method is applicable to hydrophobic systems[20]. The present study reveals that all extracts exhibit radical scavenging activity to certain extent and their distinct activities are probably derived from diversity of phytochemicals reacting uniquely with various free radicals [21]. Also, it is likely that different classes of compounds were extracted as different solvents with different polarities were used in our work. M extracts exhibited maximum antioxidant activity followed by AQ, PE and C in all samples. This was in concordance with the previous studies which suggested that extracts obtained from polar solvents were likely to show higher antioxidant activity [22].

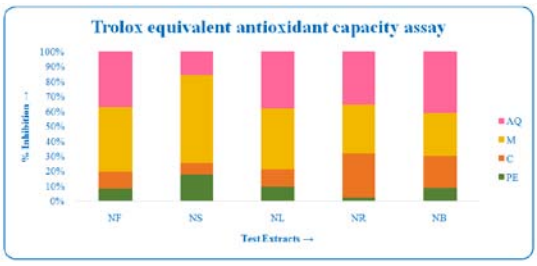

Fig. 3: The effects of different test extracts on the inhibition of the ABTS cation. {Petroleum ether (PE), chloroform (C), methanol (M), water (AQ) extracts; *Nyctanthes arbor-tristis* flowers (NF), seeds (NS), leaves (NL), roots (NR) and bark (NB); Trolox equivalent antioxidant capacity (TEAC) assay}.

Comparison of various parameters of the study displayed good correlation (Fig. 9). Significant correlation was observed between polyphenolic content and antioxidant activity, implying plausible contribution of phenolics and flavonoids to the radical scavenging activity of these plants extracts [16; 22-24]. On comparing results of two radical scavenging tests, appreciable correlation was observed. Since the basic mechanism of the assays is electron donation, the observed correlation between both assays in the present study can be explained[8].

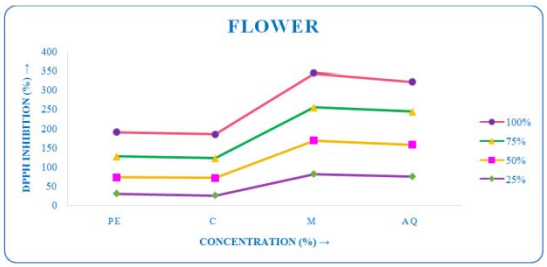

Fig. 4: The effects of *Nyctanthes arbor-tristis* flower extracts on the inhibition of the DPPH. {Petroleum ether (PE), chloroform (C), methanol (M), water (AQ) extracts; 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay}.

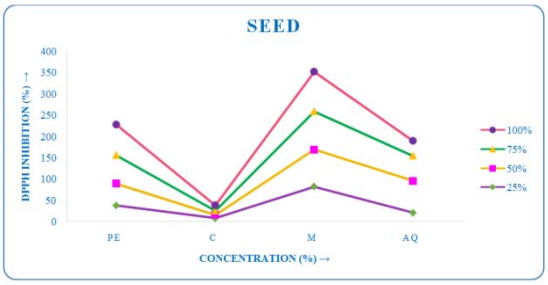

Fig. 5: The effects of *Nyctanthes arbor-tristis* seed extracts on the inhibition of the DPPH. {Petroleum ether (PE), chloroform (C), methanol (M), water (AQ) extracts; 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay}.

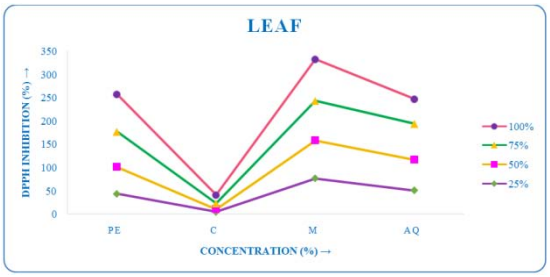

Fig. 6: The effects of *Nyctanthes arbor-tristis* leaf extracts on the inhibition of the DPPH. {Petroleum ether (PE), chloroform (C), methanol (M), water (AQ) extracts; 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay}.

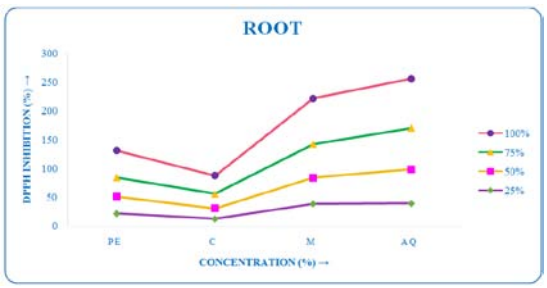

Fig. 7: The effects of *Nyctanthes arbor-tristis* root extracts on the inhibition of the DPPH. {Petroleum ether (PE), chloroform (C), methanol (M), water (AQ) extracts; 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay}.

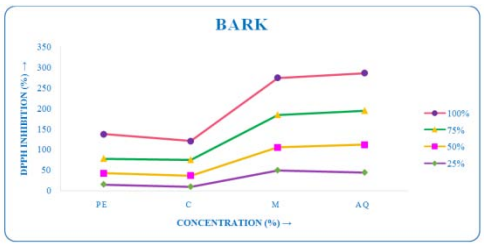

Fig. 8: The effects of *Nyctanthes arbor-tristis* bark extracts on the inhibition of the DPPH. {Petroleum ether (PE), chloroform (C), methanol (M), water (AQ) extracts; 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay}.

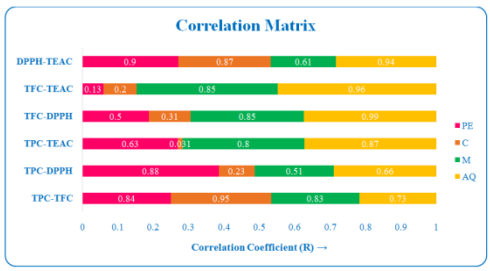

Fig. 9: Correlation matrix representing relationship between study variables {Petroleum ether (PE), chloroform (C), methanol (M), water (AQ) extracts; Total phenolic content (TPC); Total flavonoid content (TFC); 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay; Trolox equivalent antioxidant capacity (TEAC) assay}.

CONCLUSION

Global search is going on a vast scale to identify pharmacologically potent antioxidant compounds with low profile of side effects for food and health industry. Numerous sources like plants, animals and synthetic chemical preparations can be utilized for derivation of antioxidants. But safety and ethical issues are reason for concern associated with synthetic and animal-derived antioxidants, respectively. Diversity of active and therapeutically useful compounds from plants are provided by the treasure of traditional medical systems. *N. arbor-tristis* is a medicinally important plant. In the present study, antioxidant capacities along with phenolic and flavonoid content of its various parts were compared. Good correlation was observed between *in vitro* assessment of polyphenolic compounds and various antioxidant activities. This indicated that polyphenols might play important role in free radical scavenging ability of the extracts due to presence of their hydroxyl groups. The overall result of the present study certainly provides promising baseline information to ascertain the potency of the crude extracts of *N. arbor-tristis* as a potential source of natural antioxidants. These extracts emerge as a good source of natural antioxidants to be used by health and food industry. However, further investigation is suggested to identify and isolate individual components forming antioxidative system and utilize such agents with high efficacy and activity to develop their application for pharmaceutical and food industries. In addition, *in vivo* pharmacological studies should also be conducted.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENT

The authors wish to express their gratitude for financial support to the Department of Science and Technology, India through the "Innovation in Science Pursuit for Inspired Research (*INSPIRE*)-Fellowship" to Ms. Swati Vyas. Ms. Swati Vyas heartily thank her parents, Mr. Shyam Lal Vyas and Mrs. Madhu Vyas for their constant support and encouragement.

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