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Research Article

HPTLC FINGERPRINT PROFILE AND EVALUATION OF ANTIOXIDANT POTENTIAL OF FLAVONOID RICH FRACTION FROM *NYCTANTHES ARBOR-TRISTIS* L. LEAVES

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ABSTRACT

Objective: The present study was carried out to obtain the flavonoid rich extract from the leaves of *Nyctanthes arbor-tristis* Linn. for evaluation of its antioxidant activity.

Materials and Methods: In the present study, Supercritical Fluid (SCF) extraction of plant material was carried out at optimized conditions of temperature 40°C, pressure 24.51 MPa, CO_2 flow rate 2ml/min and co-solvent (ethanol) of 8.25%. Total phenolic Content (TPC) and Total Flavonoid Content (TFC) of the SCF extract were determined by Folin-Ciocalteau and Aluminium Chloride colorimetric methods, respectively. HPTLC fingerprint analysis was also done for different fractions obtained from the crude extract. Antioxidant activity of the SCF crude and ethyl acetate fractions was evaluated by DPPH and Reducing Power Assay.

Results: Extraction yield of SCF extract under optimized conditions was found to be 69.85 + 1.25. TPC and TFC were found to be 304.5 +1.70 mg GAE/g and 174.85 + 1.36 mg QE/g respectively. The optimized solvent system developed for TLC was Ethyl acetate: Methanol: Formic acid: Water [50:2:3:6]. The IC_{50} value of SCF crude and ethyl acetate fractions was found to be 62.166 + 1.86 and 55.387 + 1.26µg/mL, respectively.

Conclusion: The results of this study show that the leaf extract of *N. arbor-tristis* can be used as natural source of antioxidants.

Keywords: Antioxidant activity, Flavonoids, *Nyctanthes arbor-tristis*, Supercritical fluid extraction.

INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in medicinal plant research. Plants are a rich source of secondary metabolites which are potential sources of natural antioxidants [1]. Numerous crude extracts and pure natural compounds from leaves have been reported to have antioxidant and radical scavenging activities. Among antioxidant compounds, flavonoids and phenolics, which are widely distributed in nature, have attracted attention [2]. Flavonoids are highly effective antioxidants and their protective effects in biological systems are attributed to their capacity to scavenge free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals and inhibit oxidases [3]. Furthermore, phenolic compounds have phenolic hydroxyl groups which can dissociate to negatively charged phenolates. Dissociated phenolates can form hydrogen and ionic bonds with various proteins, which lead to disturbance of their 3-D structures and in consequence to change their bioactivity [4].

Nyctanthes arbor-tristis L. is a hardy shrub belonging to the family Oleaceae. The leaves of the plant are used in the treatment of inflammations, dyspepsia, helminthiasis, pruritus, dermatopathy, fever, etc. [5]. Several studies have been carried out to investigate the bioactivity of this plant. The alcoholic extract of the leaves of *N. arbor-tristis* was evaluated for its anti-inflammatory activity [6]. Leaf extracts were found to possess analgesic, antipyretic and ulcerogenic activities [7], hepatoprotective, immunostimulant, anti-leishmanial, anti-viral and anti-fungal activities [8]. Leaf extracts have also been found to possess good in vitro antioxidant activities [9].

The present study was undertaken to carry out HPTLC fingerprint profile of the flavonoid fractions from SCF extracts obtained from the leaves of *N. arbor-tristis* L. and to screen the SCF extract and flavonoid rich fraction for their antioxidant potential by using *in-vitro* antioxidant assays.

MATERIALS AND METHODS

Plant material

Leaves of *N. arbor-tristis* were collected from the local garden. Identification of the plant species was confirmed at Agharkar Research Institute, Pune. The leaves were separated from the stalks, thoroughly washed with tap water and rinsed with distilled water. Leaves were dried at 40°C and powdered. The powder was passed through a sieve (mesh size = 850µM) to maintain constant particle size.

Chemicals and Reagents

1,1-Diphenyl-2-picryl hydrazyl (DPPH),Quercetin and Gallic acid were purchased from Sigma-aldrich, St. Louis, USA. Folin-Ciocalteau reagent, Aluminium chloride, Trichloroacetic acid and Potassium ferrocyanide were purchased from SD-Fine Chem Ltd, Mumbai. Silica gel GF 254 TLC plates were purchased from Merck. All other chemicals and reagents used for the study were of analytical grade.

Supercritical Fluid (SCF) Extraction Apparatus

Extraction of flavonoids was done using JASCO (Japan Spectroscopic Co.) 900 series Supercritical fluid extractor/Chromatograph, which included the following: 100 ml extraction vessel, temperature control unit (JASCO C0-965), high-pressure pump (JASCO-PU-980), automated back pressure regulator (JASCO 880-81). The refrigerating coolant, L.R. grade methanol was used as a coolant. It was circulated at -5°C for cooling the SC-CO_2 extraction apparatus. Absolute ethanol (95% EtOH) acted as the co-solvent. The extraction of flavonoids from *N. arbor-tristis* leaves extract was carried out at optimized conditions of pressure at 24.51MPa, temperature at 40°C, CO_2 flow rate at 2mL/min and Co-solvent (ethanol) at 8.25% [10].

The extraction yield of final crude extract was calculated as follows:

$$\mathbf{Y_{extract}\,(mg/g) = m_{extract} \,/\, m_{herb}\ X\ 100}$$

Where $Y_{extract}$ is the % extraction yield; $m_{extract}$ is the crude extract mass (g) and m_{herb} is the extracted herb mass (g)

Phytochemical Screening of flavonoids

Presence of flavonoids in the SCF leaf extract was checked qualitatively by performing Shinoda and Ammonium test [11-12].

Estimation of Total Phenolic Content

The Total Phenolic Content (TPC) of the SCF extract was determined by Folin-Ciocalteau method with slight modifications [13]. Folin–

Ciocalteau reagent contains a metal like polytungston. Phenolics in the sample reduce the metal and change the colour from yellow to Prussian blue. The intensity of the colour is directly proportional to the phenolic content.

For the preparation of a calibration curve, 0.5 ml aliquots of (100-350µg/ml) Gallic acid solutions were mixed with 0.5ml Folin–Ciocalteau reagent and 0.5ml (7.5% w/v) sodium carbonate. Similarly, 0.5 ml SCF extract at 1 mg/ml was mixed with the same reagents as described above. After incubation at room temperature for 30 mins, the absorbance of the developed blue colour was read at 765nm (JASCO V-550 UV-Visible spectrophotometer) against reagent blank and results were calculated as gallic acid equivalents (mg/g) of extract. The concentration of total phenolic compounds in the SCF extract was determined as mg of gallic acid equivalent by using an equation that was obtained from the standard gallic acid graph. All samples were analyzed in triplicate.

Estimation of Total Flavonoid Content

Total Flavonoid Content (TFC) of the SCF extract was estimated by Aluminum Chloride Colorimetric method [14]. Basically, aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols [15]. Quercetin was used as a standard to make the calibration curve. Stock solution of quercetin (10mg/ml) was prepared in 80% ethanol and further diluted to yield a calibration range 40 to 200µg/mL. Each standard solution (0.5 mL) was separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with JASCO V-550 UV-Visible spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in the blank. Similarly, 0.5 mL of SCF extract was reacted with aluminum chloride for determination of flavonoid content as described above. All the samples were analyzed in triplicate. The total flavonoid content was expressed as quercetin equivalents (QE) in mg per g extract from a calibration curve of quercetin standard solution.

Fractionation of SCF crude extract

The phenolic rich fraction of the crude extract was obtained by successive partitioning with solvents of different polarity. The crude SCF extract was fractionated using water, hexane and ethyl acetate [16].All the fractions were collected and dried completely in an oven set at 45ºC. All the fractions were analyzed for the phenolic compounds using thin layer chromatography. 10mg of each fraction were dissolved in 1mL methanol to obtain 1mg/ml sample concentration for TLC analysis.

Thin Layer Chromatography of SCF extract

Optimization of TLC solvent system: Based on a literature survey**,** different solvent systems were tried for separation of constituents in the extract [17].Among the different systems studied, the one showing best separation for the compounds of interest in the N.arbor-tristis SCF extract was selected as the mobile phase for study.

HPTLC fingerprint analysis of SCF leaf extract and Ethyl acetate fraction

Sample preparation

10mg of each of the samples (SCF crude extract, water fraction, n-hexane fraction and ethyl acetate fraction) were weighed and dissolved separately in 1ml methanol and centrifuged at 3000 rpm for 5min.The supernatant obtained for each of the solution was used as test sample.

Sample application

10µl of test solution was loaded (8mm band width) on a 5 x 10 cm Silica gel 60 F254 TLC plate using a Hamilton syringe and CAMAG LINOMAT 5 instrument.

Mobile phase: Ethyl acetate: Methanol: Formic acid: Water [9:03:0.2:1].

Derivatising reagent: 1% methanolic 2-aminoethyl diphenylborinate.

Spot development:

The sample loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapour) along with the respective mobile phase and the plate was developed up to 85mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in the Photo-documentation chamber (CAMAG REPROSTAR 3) and the images captured at White light, UV 254nm and UV 366nm.

Derivatization and Detection

The developed plate was sprayed with the respective spray reagent and air dried. The plate was photo-documented in UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Peak analysis

The developed TLC plate was fixed in scanner stage and scanning was done at 254nm prior to derivatization. The Peak table, Peak display and Peak densitogram were noted**.**

Free Radical Scavenging Activity evaluated by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH method)

The free radical scavenging activities of all the extracts were evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) according to the previously reported method with slight modifications [18]. Briefly, a 90µM solution of DPPH in methanol was prepared, and 0.8 ml of this solution was added to 0.2 ml of the solutions of extract in methanol at different concentrations (10µg/ml - 75µg/ml). The mixtures were shaken vigorously and allowed to stand at room temperature for 1hr. Then their absorbance was measured at 517 nm using a JASCO V-550 UV-Visible spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenge the

DPPH radical was calculated by using the following formula:

DPPH scavenging effect (% inhibition) = [(A0– A1) / A0) × 100]

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance of the extract samples or reference sample. All the tests were performed in triplicate and the results were averaged.

Ferric Reducing Power Assay

Total reducing power for SCF crude extract and Ethyl acetate fraction was estimated according to the reported method with slight modifications [19]. Substances having reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which further reacts with ferric chloride to form a ferric ferrous complex. Each sample (1mL) with concentrations varying from 35-60µg/mL were mixed with 2.5 ml phosphate buffer (0.2 mM, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The reaction system was incubated at 50°C in a water bath for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5mL) and a freshly prepared ferric chloride solution (0.5 mL). The colour developed was read at 700 nm using JASCO V-550 UV-Visible spectrophotometer against a reagent blank. Increased absorbance of the reaction mixture indicated an increase in reducing power.

RESULTS AND DISCUSSION

Determination of Extraction yield under optimized SFE conditions

The extract obtained at optimum SCF conditions was dried to obtain the final extract. Under the optimized SFE conditions 40ºC, 24.51 MPa and co-solvent (ethanol) at 8.25% with CO_2 flow rate at 2 mL/min, the final % extraction yield was 69.85 + 1.25.

Phytochemical screening of flavonoids

A primary phytochemical screening of the SFE extract revealed the presence of an appreciable quantity of flavonoids in the extract [11-12].

Table 1 shows the results obtained from preliminary phytochemical analysis.

Table 1: It shows Preliminary phytochemical analysis

Phytochemical test	Color developed	Flavonoids present
SHINODA TEST	Light pink	Present
AMMONIUM TEST	Yellow	Present

Total Phenolic Content

Total phenolic content (TPC) of the SCF extract was determined by the Folin-Ciocalteau (F-C) assay using Gallic acid as the standard phenolic compound. The total phenolic content was determined using the Folin–Ciocalteau reagent. The phenolic compounds are oxidized to phenolates by the reagent at alkaline pH in a saturated solution of sodium carbonate resulting in a blue molybdenum-tungstate complex [20]. A linear calibration curve of Gallic acid, in the range of 100-350 µg/ml with coefficient of determination (R^2) value of 0.994, was obtained as shown in Figure. 1. Result of Total Phenolic Content is shown in Table 2.

Total Flavonoid Content

Total flavonoid content (TFC) can be determined in the sample extracts/ fractions by reaction with sodium nitrite, followed by the development of a colored flavonoid-aluminum complex using aluminum chloride which can be monitored spectrophotometrically at a maximum wavelength of 415 nm [21]. A linear calibration curve of quercetin used as the standard, in the range of 40 to 200µg/mL with coefficient of determination (R^2) value of 0.997, was obtained as shown in Figure 2. Result of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) is shown in Table 2.

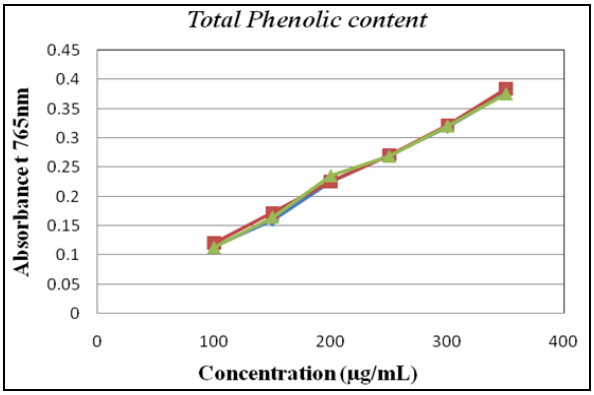

Fig. 1: It shows Calibration curve of Standard Gallic acid

R^2 values represented as mean data set for n= 3

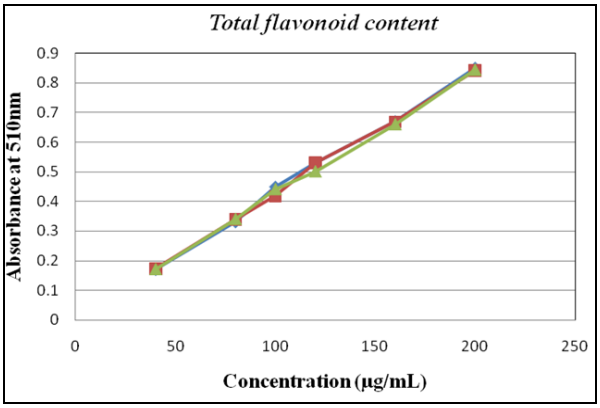

Fig. 2: It shows Calibration curve of Standard Quercetin

R^2 values represented as mean data set for n= 3

Table 2: It shows Total Phenolic and Total Flavonoid Content

Sample	Total phenolic content[a] (mg GAE/g)	Total flavonoid content[b] (mg QE/g)
SCF extract of *N.arbor-tristis*	304.5 +1.70	174.85 + 1.36

Each value is expressed as mean ± standard deviation (n = 3).

[a]Total phenolic content expressed as mg of GAE /g of extract

[b]Total flavonoid content expressed as mg of QE/g of extract.

TLC and HPTLC profile of SCF extract and flavonoids rich fraction

Proper separation of compounds was achieved using ethyl acetate-methanol-formic acid-water (9:0.3:0.2:1) as the mobile phase. HPTLC profile of the leaf extract and its corresponding HPTLC peak densitogram is given in Figure 3 and Figure 4 respectively. The results of HPTLC indicate the presence of flavonoid compounds in the extract. Yellow and orange fluorescent bands were observed at R_f 0.37 and 0.28 after spraying with 2-aminoethyl diphenylborinate reagent thus indicating the presence of flavonol group of compounds [17]. Fractionation of the crude extract resulted in the separation of compounds according to their polarity. Fractionation profile of the SCF crude extract is shown in Figure 5. Among the different fractions separated, the ethyl acetate fraction showed the presence of phenolic compounds. The ethyl acetate fraction was used as the phenolic rich fraction for further studies.

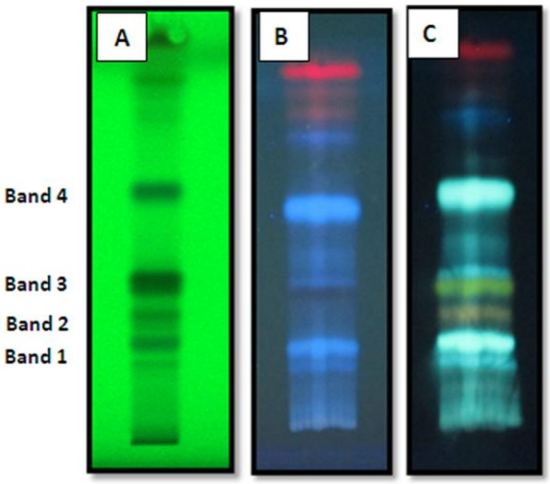

Fig. 3: It shows HPTLC profile of methanolic extract of *N. arbor-tristis* [A] Under UV 254nm; [B] Under UV 366nm-before derivatization; [C] Under UV 366nm-after derivatization.

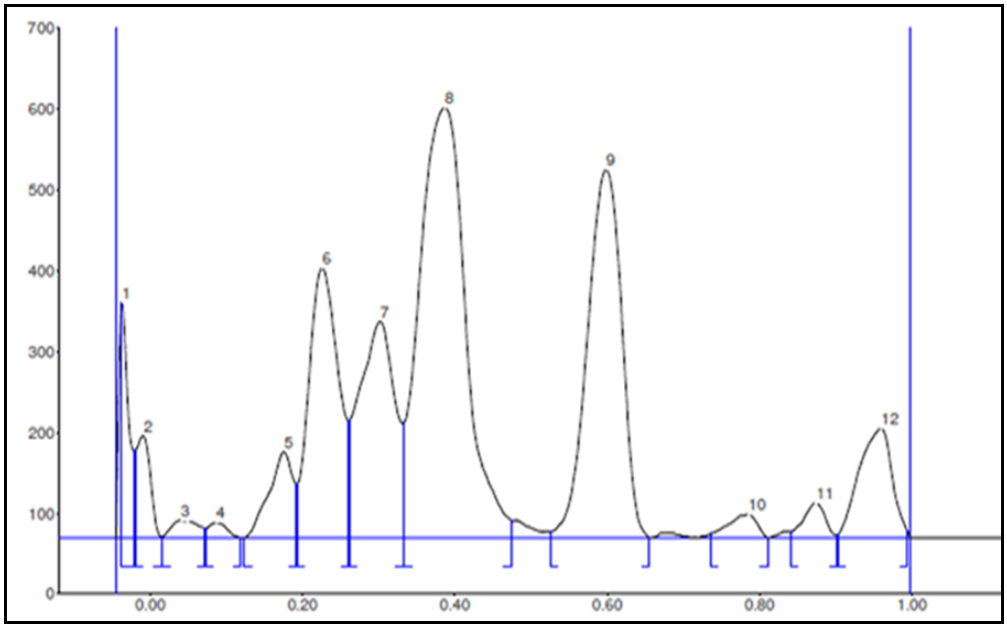

Fig. 4: It shows HPTLC peak densitogram of SCF extract of *N.arbor-tristis* scanned at 254nm

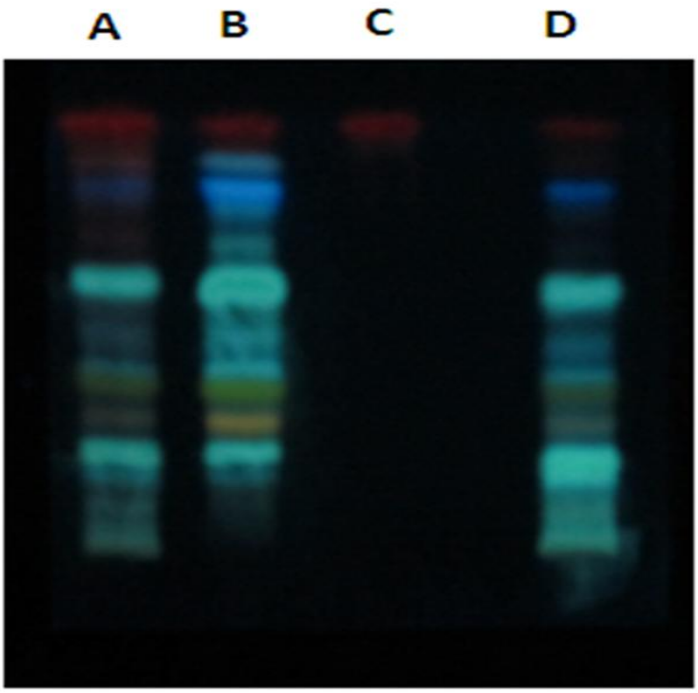

Fig. 5: It shows TLC profile of different fractions under 366nm after derivatization

[Track A- SFE crude extract; Track B- Ethyl acetate fraction; Track C- Hexane fraction; Track D- Water fraction]

Free Radical Scavenging Activity evaluated by 1, 1-Diphenyl-2-picrylhydrazyl

The SCF extract of *N. arbor-tristis* was found to be an effective free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals. Free radical scavenging activity (Figure. 6) increased with increasing concentration of the extracts in the range of 10 µg/ml -75µg/ml. Based on the results of this study, it is clear that the test plant extract has powerful *in vitro* free radical scavenging properties against a DPPH model in a concentration dependent manner. Not surprisingly, the ethyl acetate fraction showed more antioxidant activity as compared to crude extract. IC_{50} value of ethyl acetate fraction (55.387 + 1.26µg/mL) is lower than that of the crude SFE extract (62.166 + 1.86µg/mL). Results of IC_{50} value of standard and both the samples are shown in Table 5.

This could be because of the synergistic activity of phenolic compounds present in the fraction. Also this may be contributed to the fact that the hydrogen-donating compounds are more likely to be present in the polar solvents which might possibly donated hydrogen from phenolic hydroxyl groups in order to discontinue the free radical chain reaction and prevent damage from free radicals [22].

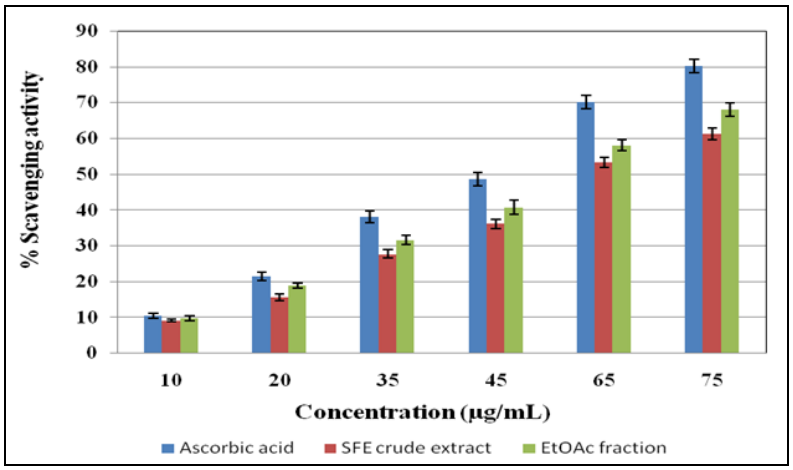

Fig. 6: It shows comparison of DPPH radical scavenging activity of different samples with standard.

Note: Bar graph represents DPPH radical scavenging activity of different samples at various concentrations. Each bar represents a mean triplicate reading + SD.

Total Reducing Power Assay

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Reducing power of SCF crude extract and ethyl acetate fraction was studied as a function of their concentration. In this assay, the yellow colour of the test solution changed to various shades of green and blue, indicating reducing power capacity of the samples.

As shown in Figure 7, a higher absorbance value indicates a stronger reducing power of the samples. The SCF crude extract as well as the ethyl acetate fraction showed concentration-dependent reducing power. However, reducing power of the crude extract was weaker than that of the ascorbic acid as well as ethyl acetate fraction.

The ethyl acetate fraction showed significant reducing power in comparison with ascorbic acid. The reducing power of the ethyl acetate fraction at50 µg/mL (0.495 + 0.08) was higher than crude extract (0.4717 + 0.01). Considering the effective reducing power of ethyl acetate fraction, the antioxidant compounds present in fraction should be able to function as good electron and hydrogen atom donors and therefore be able to terminate a radical chain reaction [23].

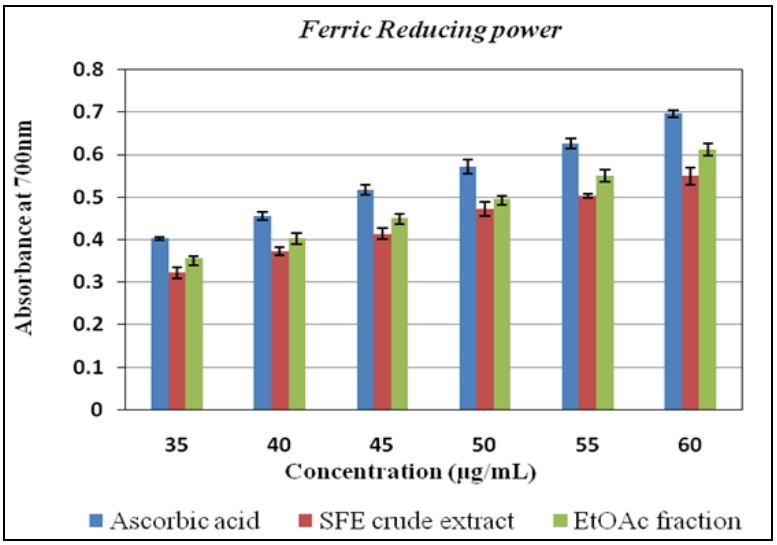

Fig. 6: It shows comparison of Ferric reducing power of different samples with standard.

Note: Bar graph represents ferric reducing power of different samples at various concentrations. Each bar represents a mean triplicate reading + SD.

CONCLUSION

Medicinal plants have complex anti-oxidative machinery that protects them against oxidative damage. Sources of these natural antioxidants are primarily phenolics and flavonoids that may occur in all products and parts of a plant. On the basis of the data obtained in the present study, it is concluded that the ethyl acetate fractions obtained from the leaves of *N. arbor-tristis* contains flavonoid components which contributes to its antioxidant activities. The presence of polyphenolic compounds in the fraction might be the major contributor of antioxidant capacity and therefore the study provides preliminary pharmacological support for utilizing them in therapeutics.

REFERENCES

1. Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J. Med. Plants Res 2011; 5(31):6697-6703.
2. Li H, Wang X, Li J. Antioxidant activities of extracts and fractions from Lysimachiafoenum-graceum Hance. Bioresource Technology 2009; 100: 970-974.

3. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, Metabolism and Structure activity relationships. J. Nutrbiochem 2002; 13:572-584.
4. Wink M. Evolutionary advantage and molecular modes of action of multi-component mixtures used in Phytomedicine. Curr Drug Metab 2008; 9:996-1009.
5. Warrier R, Nambiar VP, Ramankutty C. Indian Medicinal Plants: A Compendium of 500 species. Orient Longman Ltd, Madras; 1995.
6. Saxena R, Gupta B, Saxena K, Singh R, Prasad D. Study of anti-inflammatory activity in the leaves of *Nyctanthes arbor-tristis* Linn. An Indian medicinal plant. J. Ethnopharmacol 1984; 11: 319-330.
7. Saxena R, Gupta B, Saxena K, Singh R, Prasad D. Analgesic, antipyretic and ulcerogenic activity of *Nyctanthes arbor-tristis* leaf extract. J. Ethnopharmacol 1987; 19: 200-204.
8. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant activity of *Nyctanthes arbor-tristis* Linn. J. Ethnopharmacol 1994; 42:31-37.
9. Saxena R., Gupta B., Saxena K.and Lata S. Tranquillizing, antihistaminic and Purgative activity of Nyctanthes arbor-tristis leaf extract. J. Ethnopharmacol 2002; 81: 325.
10. Parab LS, Satam NK, Bhagwat AM, Bhoir SI. Supercritical fluid extraction and evaluation of antioxidant activity of flavonoids from *Nyctanthes arbor-tristis* L leaves. Int J Pharm Pharm Sci.2013;5 (1):256-261.
11. Sahu VK, Irchhaiya R, Shashi A, Gurjar H.Phytochemical investigation and chromatographic evaluation of the ethanolic extract of whole plant extract of *Dendrophthoe falcata* (L.F.) Ettingsh. IJPSR 2010; 1(1):39-45.
12. Edeoga H, Okwu D, Mbaebie B. Phytochemical constituents of some nigerian medicinal plants. Afr J Biotechnol 2005; 4:685-688.
13. Hung Y, Sava M, Makan SY, Chen TJ, Hong M, Huang GS. Antioxidant activity of melanin derived from tea: Comparison between different oxidative states. Food Chem 2002; 78: 233-240.
14. Chang C., Yang M., Wen H. and Chern J. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. J Food Drug Anal 2002; 10: 178-182.
15. Mabry T.J, Markham K.R., Thomas M.B. The ultraviolet spectra of flavones and flavonols. The systematic identification of flavonoids. 2nd edn. New York: Springer Verlag; 1970. 41-164.
16. Singh R, Singh B,Singh S,Kumar N,Kumar S,Arora S.Investigation of Ethyl Acetate Extract/Fractions of *Acacia nilotica* willd. Ex Del as Potent Antioxidant. Rec. Nat. Prod 2009; 3(3): 131-138.
17. Andersen M, Markham K.Flavonoids Chemistry,Bioachemistry and Applications.Taylor and Francis; 2006.
18. Patwekar F., Patwekar M., Asif M., Heroor S., Mohsin A.A. Activity guided Separation of Phytoconstituents from the Flowers of *Ichnocarpus frutescens* L. and evaluation for antioxidant property. Res J Pharmbio Chem Sci. 2010; 1(4): 318-323.
19. Jayanthi P and Lalitha P. Reducing power of the solvent extracts of *Eichhornia crassipes* (mart.) solms. Int J Pharm Pharm Sci. 2011; 3 Suppl 3:126-128.
20. Olabinri BM,Olaleve MT,Bello OO,Ehigie LO and Olabinri PF. In vitro antioxidant potentials of Mango and Pawpaw leaf extracts. J Trop Med. 2010; 5(2):40-45.
21. Bakar AM, Mohamed F, Rahmat M, and Fry J. Phytochemicals and antioxidant activity of different parts of bambangan (Mangifera pajang) and tarap (Artocarpus odoratissimus). Food Chem 2009; 113: 479–483.
22. Krishnaswamy T, Sellamuthu M, Subramaniam P. Antioxidant and free radical scavenging potential of leaf and stem bark extracts of B*auhinia malabarica R*oxb. Int J Pharm Pharm Sci 2013; 5 Suppl 1:306-311.
23. Nuria MA, Youna MH, Aalt B, Guido RMMH. Optimizing the bioactive potential of wheat bran by processing. Food and Function 2012; 3: 362-375.