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Original Article

Phytochemical and antioxidant activity of ethanolic bark extract of *Nyctanthes Arbor- Tristis Linn*

Sonu Sharma[1]*, Ayaz Ahmad[1], Seema Gupta[2]

[1]Nims Institute of Pharmacy, Nims University, Shobha Nagar, Jaipur, Rajasthan, India
[2]Gyan Vihar School of Pharmacy, Suresh Gyan Vihar University, Rajasthan, India

Abstract

Nyctanthes arbor-tristis Linn family Nyctantheaceae is widely distributed and easily available in the nature, therapeutic use of this plant is detailed in Charaka samhitha, Sushrutha samhita and other traditional systems of medicine. The seeds are cooling and astringent and are useful in vitiated condition of pitta. Seeds are useful in bilious disorders, yield fatty oil used as an illuminant. Oil from seed is used for poisonous bites, bowel complaints, epilepsy and for blackening the hair. Bark has expectorant activity and has the power of removing pains and aches. The drug is also used as hepatoprotective. The dried leaves were subjected to successive extraction using different solvent such as petroleum ether, chloroform, ethyl acetate, and methanol. These solvent extracts were subjected to a phytochemical evaluation to detect the different chemical principle present i.e. carbohydrates, protein, amino acids, steroids, glycosides, alkaloids, tannins, and phenolic compounds and to carry out chromatographic evaluation to isolate the chemical compounds. The present study was aimed at chromatographic evaluation of Nyctanthes arbor-tristis Linn leaves methanol, petroleum ether extract of leaves of Nyctanthes arbor-tristis Linn was screened in-vivo antioxidant investigations. The Antioxidant activity of the various extracts of bark of Nyctanthes arbor-tristis Linn was evaluated by various methods. In-vivo screening antioxidant shows significant activity in ethanolic extract of 100 mg/kg and 200 mg/kg dose when compared with standard drug silymarin. Thus, it may conclude that Nyctanthes arbor-tristis Linn leaves has marked antioxidant property.

Keywords: Chromatography, Antibacterial, Hepatoprotective, Expectorant.

***Corresponding Author: Sonu Sharma,** Department Pharmacognosy, NIMS institute of pharmacy, NIMS University, Shobha Nagar, Jaipur. Email address: sonu.sharma283@gmail.com

1. 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. Herbs

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are staging a comeback and herbal 'renaissance' is happening all over the globe. 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]. It is distributed throughout India, in dry areas, mostly as follows in Arid and semi-arid regions of North-western India Upper gangetic plain and on the lower range of the Western Himalayas Occurring wild in the Sub-Himalayan region from Chenab to Nepal to 1500 m Chhota Nagpur, Rajasthan, Madhya Pradesh and Southwards to Godavari [3]. The plant is a shrub or a small tree having 10 m height, young branches have angular stem. A hardy, hard shrub or a small tree, with grey or greenish white rough bark and quadrangular branches. It is essentially a warm season crop. A long period of warm and humid climate is required. It can be grown in mild climate, but is sensitive to forest. The optimum temperature requirement is 24-30 C for successful crop growth. It is mostly grown as mixed crop in kharif season [4].

The phytochemical investigation of a plant may involve authentification and extraction of the plant material; separation and isolation of the constituents of pharmaceutical Interest, characterization of the isolated compound; investigation of the biosynthetic pathway to particular compounds and the quantitative evaluation. The choice of extraction procedure depends on the nature of the plant materials and the components to be isolated. The size reduction of the dried plant materials is an important factor for extraction. If the partical size are too fine a solid cake may be produced, which will affect the flow of menstrum and will result in the formation of 'dry pockets' within the body of the material. If the materials is too coarse then interstics are formed, which leads to a speedy percolation of menstrum. This can even lead to an incomplete extraction with a need of excessive volumes of menstrum to exhaust the marc [5]. The composition of the drug or the nature of the drug i.e. hard or soft, thick or thin will affect the degree of comminution need not to great. If the materials used are hard and woody, then the sized is required to be greatly reduced while some of the substances like aloes or gum resin need only to be crushed. Materials to be powdered roots, rhizomes, bark, corms, woods. There are five officials grade of powder. The medium course or the moderately fine powders are most suitable for the purpose of extraction [6].

2. Materials and Methods:

All chemicals were of highest available purity and were procured from E. merc Mumbai, India, Himedia laboratories, Mumbai, India and SD fine chemicals, Mumbai, India.

Collection and authentication of plant

The dried gumy bark of Nyctanthes arbor-tristi was collected in the day time locally from Anand District, Gujarat, India, in the month of September 2009. The plant was positively authenticated by department of Botany, University of Pune, India. A voucher specimen is deposited in the Department of botany, university of Pune for preparation of Plant material for extraction.

The collected plant materials i.e. bark of Nyctanthes arbor-tristis Linn was washed, cut into small pieces and were allowed to

dry in the shade, then they are pulverized in mixer grinder to coarse powder and passed through mesh size 40 sieve.

Preparation of Plant Extracts-: 7

Requirements:

(a) Dry coarse powder of bark of Nyctanthes arbor-tristis Linn
(b) Large cork-stopper glass bottle with wide mouth
(c) Petroleum ether (60-80C)
(d) Chloroform
(e) Ethanol

Procedure:

Preparation of Petroleum ether extract: The shade dried, coarse powder of the stem bark of Nyctanthes arbor-tristis Linn (300gm) was packed well in a soxhlet apparatus and extracted with petroleum ether (60-80°C) until the extraction was completed which was confirmed by the colour of the siphoned liquid. The extract was filtered while hot and the resulting extract was distilled in vacuum in order to remove the solvent completely and subsequently dried in a dessicator. The extract was weighed and calculated the percentage yield in terms of air dried material.

Preparation of chloroform extract: The marc left after petroleum ether extract was dried completely in hot air oven below 50°C and then packed well in soxhlet apparatus and extracted with chloroform until the extraction was completed. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure to remove the solvent completely and dried in a dessicator. Weighed the obtained extract and calculated its percentage yield in terms of air dried powdered crude material.

Preparation of ethanol extract: The marc left after chloroform extraction was dried in hot air oven below 50°C and packed well in soxhlet apparatus and extracted with ethanol until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely and dried in a dessicator. Weighed the extract and calculated its percentage in terms of air dried powdered crude material.

Fluorescence studies

Evaluation of different extracts based on the fluorescence in day light is likely to be unreliable due the weakness of the fluorescence effect. Some extracts may show fluorescence when the sample is exposed to ultraviolet radiation. Hence, the crude extracts were examined for the characteristic fluorescence and reported as in Table 2.

Phytochemical Analysis of acetone and ethanol extract of bark of Nyctanthes Arbor-Tristis Linn:

Preparation of reagents:

Millions reagent: Dissolve 1 gm of mercury in 9 ml of fuming nitric acid, keeping the mixture well closed during the reaction. When the action is complete, add equal volume of distilled water.

Ruthenium red: Dissolved 0.008 g of ruthenium red in 10 ml of 10 % solution of lead Acetate.

Sudan red III: Dissolved 0.04 of Sudan red in 20 ml of alcohol (90%) and 20 ml Glycerin.

α-Napthol solution: 1gm of 1-napthol is dissolved in solution of 6 gm of Sodium

Hydroxide and 16 gm of anhydrous sodium carbonate in 100ml of water.

Mayer's reagent: 1.36 gm of Mercuric chloride is dissolved in 60ml of distilled water. And 5 gm of Potassium iodide is dissolved in other 20 ml of water. Both the solution is mixed and Volume is adjusted to 100ml with distilled water.

Dragendorff' reagent: Boil 14 gm of sodium iodide with 5.2 g basic bismuth carbonate in 50 ml glacial acetic acid for few minute. Allow it to stand overnight and filter off the sodium acetate crystals.

Fehling solution B: Dissolved 173 g of potassium sodium tartarate and 50 gm of sodium hydroxide in distilled water and make volume up to 500 ml [8,9].

Qualitative phytochemical analysis: All the extracts obtained by the successive extraction of the powdered bark of Nyctanthes arbor- tristis Linn. With petroleum ether extract, chloroform extract and ethanol extract were subjected to various qualitative tests for the identification of various plant constituents present in this species [10, 11, 12].

1. **Test for alkaloids**:

Dragendorff's test: To 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Mayer's test: To 1 ml of extract add 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

Hager's test: To 1 ml extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow colored precipitate indicates the presence of alkaloids.

Wagner's test: To 1 ml of extract add 2 ml of Wagner's reagent (iodine in potassium iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

2. **Test for saponins**

To alcoholic extract and add 20ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. Appearance of one cm (or more) Layer of foam indicates the presence of saponin.

3. **Test for glycosides:**

Legal's test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

Baljet's test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

Keller-Killiani test: 1 gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered and add to the filtrate, 10ml of water and 0.5m1 of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening on standing.

Borntrager's test: Add a few ml of dilute sulphuric acid to 1 ml of the extract solution. Boil, filter and treat the filtrate with chloroform. The chloroform layer is treated with 1ml of ammonia. The formation of red colour in the ammonical layer shows the presence of anthraquinone glycosides.

4. Test for carbohydrates ana sugars:

Moliseh's test- To 2ml of the extract, add l ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of carbohydrates.

Fehling's test: To 1 ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

Benedict's test: To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

5. Test for Phenolic Compounds and Tannins

1. Taken a little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

2. To 1 ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colored product shows the presence of tannins.

3. A little quantity of extract is treated with potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

4. To the test extract, add strong potassium dichromate solution, a yellow coloured precipitate indicates the presence of tannins and phenolics.

6. Test for Flavonoids

1. The drug taken in alcohol or water along with a few ml of ammonia is viewed in UV and visible lights, formation of fluorescence indicates the presence of flavonoids.

2. A little quantity of the extract is treated with amyl alcohol, sodium acetate and ferric chloride. Solution turns yellow and disappears on addition of an acid. This indicates the presence of flavonoids.

3. Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl gives intense cherry red colour indicates the presence of flavonones. Orange red colour indicates the presence of flavonols.

4. The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.

5. The extract is treated with concentrated H_2SO_4, formation of yellow or orange colour indicates flavones.

6. The alcoholic and aqueous extract is treated with 10% sodium chloride; formation of yellow colour indicates the presence of coumarins.

7. Test for steroids and sterols:

Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance, of bluish-green colour shows the presence of sterols.

Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H2SO4 Formation of bluish red to the colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

8. Test for proteins and amino acids:

Biuret test: Add 1 ml of 40% sodium hydroxide solution and 2 drops of 1% $CuSO_4$ solution till a blue colour is produced, and then add 1 ml of the extract. Formation of pinkish or purple violet colour indicates the presence of proteins.

Ninhydrin test: Add two drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue colour reveals the presence of proteins or peptides or amino acids.

Xanthoproteic test: To 1 ml of the extract, add l ml of concentrated nitric acid. A white precipitate is formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange colour indicates the presence of aromatic amino acids.

Millon's test: l ml of test solution is made acidic with sulphuric acid and add Millon's reagent and boil this solution. A yellow precipitate indicates the presence of protein.

9. Test for triterpenoids: Noller's Test: Dissolve two or three granules or tin metal in 2ml thionyl chloride solution. Add 1 ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids.

6. **10. Test for fixed oils and fats:**

Spot test: Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.

Saponification test: To l ml of the extract, add few drops of 0.5 N alcoholic potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

11. Test for gums and mucilage: Add about 10 ml of aqueous extract slowly to 25m1 of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

Thin Layer Chromatography (TLC): The technique of thin layer chromatography closely resemble those of column and paper Chromatography. In thin layer chromatography, partition, however, occur on a layer of finely divided adsorbent, which is supported on a glass plate. This chromatography using thin layers of an adsorbant held on a glass plate or other supporting medium is known as thin layer chromatography [13].

Feature and applicability of TLC: All chromatography principles functioning in solid liquid, liquid system are also applicable to thin layer chromatography. Adsorption chromatography has widely been used, but ion exchange, partition and reversed phased partition can also be applied in thin layer technique. The choice of the chromatographic principle is determined by the chemical nature of the compounds to be resolved and by the desired pattern of fractionation.

TLC analysis of various extracts of powdered bark of Nyctanthes Arbor Tritis Linn is given in table no. 4.

Procedure: 100gm of silica gel G was weighed and made into a homogenous slung

with approx. 200gm of sufficient distilled water to form slurry. Then the slurry was poured into TLC glass plates by spreading technique and the thickness of silica gel layer on TLC plate was adjusted to about 0.25 mm thickness. The coated plates were allowed to dry in air and activated by heating in hot air oven at 100-105°C for 1 hour.

The extracts were prepared with the respective solvent like petroleum ether, chloroform and ethanol and made up to 10ml in different test tubes. Then the extracts were taken in a capillary tube and it was spotted in glass plates coated with silica gel G. The plates were developed in TLC chamber previously saturated with different solvent systems

For isolation and resolution of spots of the three extracts the following solvent systems were selected by trial and error method.

(1) n-hexane: ethylacetate : (70:30)

(2) n-hexane: ethylacetate : (60:40)

(3) ethylacetate : methanol : water (60:20:20)

The different spots developed in each solvent system were identified by means of UV light and the R_f values were correspondingly calculated and presented in Table 5.

Column chromatography:

Principle: Each compound in a mixture will have a particular solubility in the solvent and a particular tendency to be absorbed by the solid adsorbent. Mostly no two compounds behave exactly alike in these respects. This principle is utilized in column chromatography.

Details of Column chromatography

Adsorbant: Silica gel (for column chromatography 60-120 #)

Eluent : Petroleum ether to distilled water in gradation.

- Length of column: 60 cm
- Diameter of column: 3.5 cm
- Amount of Ethanolic extract used: 5 gm
- Length of column packed: 40 cm
- Rate of Elution: 30 drops per minute
- Fractions collected: Each of 100 ml

Procedure: The column with the cotton plug is filled with the sufficient silica gel (60-120 #) up to 40 cm in the given column height of 60 cm and 3.5 cm width. The column was carefully packed and uniformly filled with silica gel, by tapping the side of the column. Then the ethanolic extract of powdered bark of *Nyctantlies arbor - tristis Linn.* was charged on column and eluted with different solvents ranging from non-polar to polar at the rate of 30 drops per minute. Each fraction was collected in the volume of 100 ml with different solvent ratio as given in Table 6

Spectral Studies:-

(a) UV absorption spectroscopy: The measurement of absorption of ultraviolet and visible radiation provides a convenient means for the analysis of numerous inorganic and organic species. The wavelength in UV region is usually expressed in nm that is 200 - 400nm.

The UV spectra of isolated fraction 1 and fraction 2 were done at Cadila, Ahmedabad. The sample solution was prepared in benzene and the same

benzene was used as blank. UV scan was done between 200 - 400 nm and the speed of instrument scanning was set as fast. The peak absorbance obtain for both fractions are tabulated as follows in table no. 7.

Acute oral toxicity studies [14, 15]

Procedure: Five Wistar Albino rats of either sex having weight 180-230 gm were used for the study. The animals were obtained from the animal house of Cadila pharmaceuticals, Ahmedabad. Fixed dose levels of 50, 100, 200, 500, 1000 were given initially to allow identification of a dose producing evident toxicity for the ethanolic bark extract of *Nyctanthes arbor - tristis Linn.* After giving the dose the toxic signs were observed within 48 hours, Food was withheld for a further period of 3-4 hours after administration of drug. The further 2000 mg/Kg was administered after the last dose and observed for the mortality.

As most of the crude extracts possess LD more than 2000 mg/Kg (LD_{50} >2000 ^{m}g/kg). Body weight of the rats before and after administration was noted and any changes in skin, fur, eyes, mucous membranes and also respiration and behavior pattern were observed. Also signs of tremors, convulsions, salivation, diarrhea, sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted, if any.

3. Observation and Results

There were no any considerable changes in body weight before and after administration and signs of toxicity were not present. Thus the LD_{50} of the ethanolic bark extract as per OECD guidelines - 420 is greater than 2000mg / Kg (LD_{50}> 2000 mg/kg)

Antioxidant activity [16, 17] **Methodology:**

Homogenate Preparation: The frozen liver samples were homogenized in The-HCL or phosphate buffer solution to give a 20% homogenate. To measure lipid peroxidation levels, homogenate was centrifuged at 1700 rpm and 4°C for 10 min. For assay of catalase activity the homogenate was centrifuged at 3000 rpm and 4°C for 15 min, and then diluted to 0.5%. After centrifugation at 3000 rpm for 15 min, the supernatant was again centrifuged either at 10000 rpm for 1 min and diluted to 2% for measurement of glutathione peroxides activity or at 30 000 rpm for 10 min before assessment of tissue superoxide dismutase activity with 20% ethanol.

Measurement of tissue lipid peroxidation Levels:

The extent of lipid peroxidation was quantified by measuring the thiobarbituric acid reactive substance (TBARS) – malonaldehyde produced during peroxidation of lipids.

Procedure:

To 1ml of the homogenate 1.5m1 of TCA in HC1 was added and was allowed to stand for 15 minutes at room temperature. The tube was centrifuged and to the supernatant 1.5 ml of TBA solution was added and was heated in a boiling water bath for 15 minutes. After cooling to room temperature, 3 ml of chloroform was added and the mixture was shaken vigorously for 3 minutes and centrifuged for 10 minutes at 1500 rpm. The absorbance of the chromophore was measured at 530 nm. A standard curve was constructed using TEP hydrolysed MDA containing 5-30 nm. The level of lipid peroxidation was expressed as

nano mole of malonaldehyde per mg of protein.

Assay of superoxide dismutase (SOD)

The antioxidant efficacy was measured by the assay of superoxide dismutase

Procedure:

To 150 µl of the homogenate 1.8m1 carbonate buffer, 0.7 ml of distilled water and 400µl of epinephrine were added and mixed well and the increase in absorbance at 480 nm was measured spectrophotometrically. Auto-oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. Activity was expressed as units/min/mg of protein.

Assay of glutathione peroxidase

Glutathione peroxidase was assayed by the method of Rotruck *et al.,* (1973) with some modification.

Procedure:

To 100 µl of the homogenate 0.4 ml of buffer, 0.2 ml sodium azide, 0.2 ml EDTA, 0.2 ml of hydrogen peroxide and 0.2 ml of reduced glutathione were added and made up to a volume of 2ml with water. The tubes were incubated at 37°C for 10 minutes. 1 ml of trichloroacetic acid was added to terminate the reaction. The reaction mixture was centrifuged and to the supernatant, 8 ml of disodium hydrogen phosphate was added followed by 1 ml of trichloroacetic acid was added. 1 ml of DTNB was added just prior to the analysis. The absorbance was read at 412nm against blank which contained only 8 ml of the phosphate solution and 1 ml of DTNB reagent. A standard graph was constructed using 20 to 100µgm of reduced glutathione. The activity was expressed as units/ mg of protein.

Assay of catalase:

Antioxidant enzyme catalase was assayed by the method of Srnha, (1972).

Procedure:

To 1 ml of the phosphate buffer taken in each of the four test tubes, 0.1 ml of the homogenate was added. To this 0.5 ml of hydrogen peroxide was added. The reaction was stopped at 15, 30, 45 and 60 seconds by the addition of 2ml of dichromate acetic acid reagent. The tubes were boiled for 10 minutes, cooled and read at 610nm. A `zero time' run was also made simultaneously by adding the dichromate acetic acid reagent before the addition of hydrogen peroxide. This activity was expressed as micromoles of hydrogen peroxide utilized/min/mg of protein.

Results and Discussion:

As the amount of catalase, superoxide dismutase, Glutathione Peroxidase decrease in presence of CCl_4 showing increase in free radicals and utilization of these enzymes and increase in TBARS due to leakage show toxicity induced by CCl_4. The amount of catalase, superoxide dismutase, Glutathione Peroxidase increases when the plant extracts of 100 mg/kg and 200 mg/kg showing protective potential against free radicals. Similarly, decrease in the amount of TBARS in presence of plant extract showing protective potential of plant extract against free radicals. Silymarin (25mg/kg) were used as standard drug. When the effect of plant extract was compared with standard drug, it is found that higher dose of plant extract 200 mg/kg show better result as compared with lower dose of plant extract 100 mg/kg.

Table- 1 Percentage yields of acetone and ethanol extract of bark of *Nyctanthes Arbor-Tristis Linn*

S. No.	Extracts	% Yield
1	Petroleum ether extract	0.52
2	Chloroform extract	0.43
3	Ethanolic extract	28.0

Table 2 Qualitative phytochemical analysis of various extracts of powdered bark of *Nyctanthes Arbor–Tristis Linn*

Plant constituents	Petroleum Ether extract	Chloroform extract	Ethanol extract
Alkaloids	-	+	+
Saponins	-	-	-
Glycosides	-	-	+
Carbohydrate	-	-	+
Tanins & Phenolic compounds	-	-	+
Flavonoids	-	-	+
Phytosterols	+	-	-
Proteins & amino acids	-	-	-
Triterpenoids	-	-	-
Fixed oil & fats	+	-	-
Gums & Mucilage	-	-	+

Present (+) **Absent** (-)

Table 3: Fluorescence study of various extracts of powdered bark of Nyctanthes Arbor-Tristis Linn

Sr. No.	Extracts	Day Light	UV Light (254 nm)
1	Petroleum ether extract	Brown	Light Green
2	Chloroform extract	Yellowish Brown	Dark Green
3	Ethanolic extract	Yellowish orange	Light Green

Table no 4: TLC of various extracts of powdered bark of Nyctanthes Arbor-Tritis Linn

Sr. No.	Extracts	Solvent system	No. of spots	Colour of spots	R_f values
1	Petroleum ether extract	n-hexane: ethylacetate (70:30)	2	Light blue Blue	0.24 0.52
2	Chloroform extract	n-hexane: ethylacetate (60:40)	1	Bluish green	0.82
3	Ethanolic extract	ethylacetate: methanol: water (60:20:20)	3	Blue Dark blue Dark blue	0.20 0.62 0.81

Table 5 Column chromatography of ethanolic extract of powdered bark of Nyctanthes Arbor- Tristis Linn.

S. No	Solvent Fraction	No. of Spots	R_f Value of Spots	Colour of Spots
1	Petroleum ether (100)	0	-	-
2	Pet. Ether : Benzene(75:25)	0	-	-
3	Pet. Ether : Benzene(50:50)	0	-	-
4	Pet. Ether : Benzene(25:75)	0	-	-
5	Benzene (100)	0	-	-
6	Benzene : Chloroform (75:25)	1	0.62	Blue
7	Benzene : Chloroform (50:50)	1	0.61	Light Blue
8	Benzene : Chloroform (25:75)	1	0.64	Dark Blue
9	Chloroform (100)	1	0.61	Light Blue

10	Chloroform : Ethyl acetate (75:25)	0	-	-
11	Chloroform : Ethyl acetate (50:50)	0	-	-
12	Chloroform : Ethyl acetate (25:75)	0	-	-
13	Ethyl acetate (100)	0	-	-
14	Ethyl acetate : Methanol (75:25)	1	0.82	Bluish Green
15	Ethyl acetate : Methanol (50:50)	1	0.81	Bluish Green
16	Ethyl acetate : Methanol (25:75)	1	0.82	Bluish Green
17	Methanol (100)	0	-	-
18	Methanol : Water (75:25)	1	0.21	Light Blue
19	Methanol : Water (50:50)	0	-	-
20	Methanol : Water (25:75)	0	-	-
21	Water (100)	0	-	-

Table No. 6 UV Absorption spectroscopy of ethanolic bark extract of *Nyctanthes Arbor- Tristis Linn*

Sample	λ max. (nm)	Absorbance
Fraction 1	280.00	0.26738
Fraction 2	275.00	1.78985

Table No.7 Antioxidant activity of ethanolic bark extract of *Nyctanthes Arbor- Tristis Linn*

Treatment	Catalase (U/mg liver protein)	Superoxide Dismutase (U/mg liver protein)	Glutathione Peroxidase (U/mg liver protein)	TBARS (nmole/mg liver protein)
Normal	295.07±19.1	77.81±6.21	0.965±0.030	1.28±0.389
CCl_4 control	164.75 ± 6.78	31.31±0.67	0.737±0.053	1.69 ±0.14

Plant Extract (100 mg/kg)	265.58 ±6.81*	57.10±0.73*	0.573±0.021*	1.62 ±0.11*
Plant Extract (200 mg/kg)	269.27 ±7.54*	68.45 ±4.63*	0.874±0.074*	1.77 ±0.12*
Silymarine (25mg/kg)	270.3 ±9.3*	68.52 ±7.1*	0.98 ±0.05*	1.88 ±0.14*

Data are expressed Mean ± S.E., n = 6. ,* $p < 0.01$ Vs Control by student `t' test.

Conclusion

Phytochemical studies were focused on preparation of Petroleum ether, Chloroform and ethanolic extract of bark powder of *Nyctanthes arbor - Tristis* linn. All three extracts were studied to identify the presence of specific phytoconstituents in them. It was identified that Petroleum ether extract contains fixed oils and fats. Whereas chloroform extract showed presence of only alkaloids and ethanolic extract shows maximum phytoconstituents positive results viz alkaloids, glycosides, carbohydrates, tannins, gums and mucilage and flavonoids. Thin layer chromatographic investigations of all three extracts showed maximum presence of phytoconstituents in ethanolic extract and reported Rf relevant with various phytoconstituents. Coloum chromatographic analysis of ethanolic extract was carried with various solvents basis on polarity and fractions were collected for spectrophotometrically identification. UV spectroscopic analysis of two collected fractions was carried and relevant absorbance was noted.

In- vivo screening antioxidant shows significant activity in ethanolic extract of 100mg/kg and 200mg/kg dose when compared with standard drug silymarin. It can be concluded from the present work that bark of *Nyctanthes arbor - tristis* Linn shows significantly positive antioxidant activity when compared with silymarin as standard drug. The activity may be due to presence of alkaloids, flavonoids and glycosides in ethanolic extract.

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