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Phytochemical screening and TLC fingerprinting of selected medicinal plant used for antilithiatic activity

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Abstract--The purpose of this study to investigate phytochemical screening and TLC fingerprinting of selected medicinal plant used for antilithiatic activity. Phytochemical tests were performed on plant extracts to determine the presence of several chemical elements such as Glycoside, Terpenoid, Flavonoids, Tannins, and other compounds that are responsible for the biological activity of the plant extract. To that end, ethanolic extracts of *Nyctanthes arbor-tristis* Linn. leaves are used for the chemical assays to be conducted primarily for the identification of various chemical components The most effective solvent system for TLC of *Nyctanthes arbor-tristis* Linn. leaves was discovered. Chloroform, benzene, and formic acid are all components of the ethanolic extract (50:40:10). *Nyctanthes arbor-tristis* Linn. leaves have received some TLC. In the presence of six different compounds with various R_f values in different colours, employing the iodine vapour as a detecting reagent, the ethanolic extract shows the presence of six compounds in the extract with different R_f values in different colours. These observations provided conclusive evidence that the leaves of *Nyctanthes arbor-tristis* Linn. contaminated was the source of the plant's existence Indications for its usage as a medicinal plant include the fact that it is a potent source of a number of chemicals that have therapeutic significance. This can be investigated in greater depth for the purpose of isolating and discovering active biochemical substances that have the potential to be used therapeutically.

Keywords---*Nyctanthes arbor-tristis* Linn., Phytochemical Screening, TLC.

Introduction

Urolithiasis is a complex process that is caused by an imbalance between promoters and renal inhibitors ¹. Even following technical breakthroughs in the medical practise, humanity continues to suffer from the formation and proliferation of renal calculus. Calcium oxalate and calcium phosphate constitute almost 80 percent of these calculations. Urinary calculations may induce blockage, hydronephrosis, urinary tract infection and haemorrhage ². The production of kidney stones is a complex process which occurs from a series of physicochemical events, including supersaturation, nucleation, growth, aggregation and retention in kidney tubules ³. Surgery, lithotripsy and local calculus disruptions are extensively utilised for the removal of calculus with high power laser. However, recurrence is fairly prevalent in these operations ⁴. The recurrence rate without preventative treatment is about 10% in 1 year, 33% in 5 years, and 50% in 10 years. Several medications are used to prevent recurrence, including thiazide diuretics and alkaline citrate, but empirical evidence for their effectiveness is less compelling ⁵. Therefore, the hunt for effective antilithiatic medications has acquired enormous promise without or minimum side effects from natural sources. In contrast, traditional medicinal products have replaced many ailments and have also contributed further information regarding disease causation ⁶. *Nyctanthus arbor-tristis* Linn. is a small frightened ornamental tree popular throughout the country for its fragrant, flowers. *Nyctanthus arbor-tristis* Linn. is often referred to as Night jasmine or parijatas.

Plant name : *Nyctanthus arbor-tristis* Linn.

Family : Oleaceae

Vernacular names

Tamil	:	Pavala- malligai, Manjhapu
English	:	Night jasmine, Coral jasmine
Hindi	:	Harsinghar, Seoli, Sihau
Sanskrit	:	Parijatha, parijatah, sephalika
Malayalam	:	Mnnapu, Pavizhamalli, Parijatak
Telugu	:	Kapilanagadustu, Pagadamalle
Kannada	:	Goli, Harsing, Parijata
Marathi	:	Kharbadi, Kharassi, Khurasli.
Gujarathi	:	Jayaparvati
Punjabi	:	Harsinghar

Taxonomical name

Kingdom	:	Plantae
Order	:	Lamiales
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Family	:	Oleaceae
Genus	:	Nyctanthes
Species	:	Arbortristis



Fig. 1: Leaves of *Nyctanthus arbor-tris* Linn.

Morphology

Colour: Light to dark green

Odour: Indistinct

Taste: Bitter and astringent

Leaf: Simple, 5-14 cm long, 2.5-7.5 cm wide, ovate, acute

Margin: Entire or distinctly toothed Base and Round

Venation: Reticulate, lateral vein 3-6 pairs

Phytoconstituents of *Nyctanthus arbor- tristis* leaves

Three new benzoic esters of Loganin and 6- β -hydroxy loganin, namely Arborside-A, Arborside-B, and Arborside-C were found to be present in the leaves. From leaves 10-Benzoylnyctanthoside named as Arborside-D were isolated. Other iridoid glycosides that were reported are 6, 7-Di-O-benzoyl nyctanthoside, 6-O-transcinnamoyl 6- β -hydroxy loganin and 7-O-trans cinnamoyl-6- β -hydroxy loganin from the leaves. A phenyl propanoid glucoside Des rhamnosyl verbascoside was reported from the leaves. Leaves also contain the alkaloid Nyctanthine along with Mannitol, β -Amyrin β -Sitosterol, Hentriacontane, Benzoic acid, Astragalol, Nicotiflorin, Oleanolic acid, Nyctanthic acid, Friedelin and Lupeol.

Traditional uses

The *Nyctanthus arbor-tristis* Linn. leaves are used mostly in Ayurveda. In Hindu mythology, the Parijatha is said to be one of the five great trees of Devaloka. Different components of *Nyctanthus arbor-tristis* Linn. are utilised in the remedies system Ayurveda, Sidda and Unani. Treatment of a range of ailments such sciatica, persistent fever, rheumatism and internal worm diseases, as well as laxative, diaphoretic and diuretic treatment. Leaves are mainly utilised in spleen enlargement. Blood paste is blended with sweetness for blood pressure, diabetes and fever treatment. The decoction of the leaves is primarily employed in the treatment of arthritis, malaria, intestinal worms, stubborn sciatica, cholagogue and laxative by ayurvedic doctors. Appetite loss, stacks, liver diseases, diarrhoea, intestinal worms, chronic fever, persistent sciatics, rheumatism and rigorous fever are treated with leaf juice. The leaf juice extracted functions as a

cholagogue, laxative and mild bitter tonic. Children are given little sugar as a treatment for digestive problems.

Materials and Methods

Collection and identification of plant materials

The leaves of *Nyctanthus arbor-tris* Linn. available locally were collected from Park, New Delhi. The voucher specimens had been submitted and preserved in herbarium for future reference.

Extraction of plant materials

After collecting and drying the leaves of *Nyctanthus arbor-tris* Linn. at room temperature in the shade, the powder was treated to size reduction in order to obtain a coarse powder with the necessary particle size. After that, it is pulverised and passed through a mesh size of 40 before being stored in airtight jars. Following the powdering of the materials, they were treated to a series of extractions. The cold maceration method was used to extract each (1kg) powdered medication with methanol and water separately for 7 days, followed by a wash step. In a rotary evaporator under reduced pressure, the extracts were filtered and the solvents were evaporated to obtain the dry extract, which was then concentrated. In order to conduct subsequent studies, the yield of the dry extracts was estimated and then kept in desiccators for use later on.

Phytochemical screening:

Phytochemical screening were performed using standard procedures ⁷⁻⁸.

Anthocyanins and Anthocyanidins:

When approximately 2 ml of aqueous extract is put to 2 ml of 2N HCl and ammonia, a pink red colour appears that quickly turns blue violet, indicating the presence of anthocyanins and anthocyanidins.

Anthracene glycosides:

When the sample (5 ml) is put to ammonium hydroxide (25 percent), the result is a crimson colour, which shows the presence of anthracene glycosides in the sample.

Coumarins:

The presence of coumarins is shown by the formation of a yellow colour after the addition of 3ml NaOH (10 percent) to 2ml of extract.

Saponin:

Froth test: Froth formation by shaking 1 ml solution of sample in water in a tube reveals saponin.

Phenolic:

Ferric Chloride test: Extracts were treated with few drops of 5% acidified ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Carbohydrates:

Molisch's test: Treat the test solution with few drops of alcoholic alpha naphthol. Add 0.2 ml of conc. H_2SO_4 slowly through the sides of the tube, a purple to violet ring appears at the junction. Proteins:

Xanthoprotein test: Yellow colour developed when extracts were treated with few drops of conc. nitric acid indicating the presence of proteins.

Alkaloids:

Meyer's test: To 1ml of each of the sample solution few drops of Meyer's reagent (potassium mercuric chloride solution) was added. Formation of cream white precipitate indicates the presence of alkaloids.

Wagner's test: To few ml of each of the sample solution, Wagner's reagent (iodine in potassium iodide) was added, which resulted in the formation of reddish brown precipitate indicating the presence of alkaloids.

Cardiac glycosides:

Kellar Kiliani test: Conc. H_2SO_4 (1 ml) was taken in a test tube then 5ml of extract and 2ml of glacial acetic acid with one drop of ferric chloride were added which results in formation of a blue colour.

Sterols and Terpenoids:

Liebermann-Buchard test: Samples were treated with few drops of acetic anhydride, boiled and cooled. Conc. H_2SO_4 from the sides of the test tube was added shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids and formation of deep red colour indicates the presence of terpenoids.

Salkowski test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. H_2SO_4 , shaken and allowed to stand. Appearance of red colour at the lower layer indicates the presence of sterols and yellow colour at the lower layer indicates the presence of terpenoids.

Flavonoids:

Jone's Test: To small amount of sample dissolve in 1 ml of acetone, 2 ml of 10% aq $K_2Cr_2O_7$ and 6 ml of 6 M H_2SO_4 . A blue green colour indicates the presence of flavonoids.

Tannins:

About 1% lead acetate was added 2ml of extract. A yellowish precipitate shows the presence of Tannins.

Terpenoids:

Presence of Terpenoids was showed by development of blue green colour after adding conc. HCL: Phenol (1:1).

Amino acids:

Ninhydrin test: To 1 ml of sample boiled with 0.1% acetone solution of ninyhydrin, appearance of violet colour shows the presence of amino acids.

Starch: Extracts were treated with lugol solution (1g iodine + 2g potassium iodide) and water, appearance of blue colour shows the presence of starch.

Thin layer chromatography:

Thin Layer spread as a thin layer on a rigid supporting plate; & the mobile phase, a liquid is allowed to migrate across the surface of the plate. 9-10

The ethanolic extract of powdered leaves of *Nyctanthes arbor-tristis* Linn. was subjected to thin layer chromatography studies, to find the presence of number of compounds which support by the chemical test.

Results and Discussion**Phytochemical screening of plant materials:**

The extracts were phytochemically checked and the findings are shown in Table 1.

Table 1: Preliminary photochemical analysis of leaves of *Nyctanthes arbor-tristis* Linn.

Name of Tests		Pet. ether	Ethanolic Extract
Anthocyanine & Anthocyanidine		+ve	-ve
Anthracene glycoside		+ve	-ve
Coumarins		+ve	+ve
Saponins	Foam test	-ve	-ve
Phenolics		+ve	-ve
Carbohydrates	Molish test	-ve	-ve
Alkaloids	Mayer's test	-ve	-ve
	Wagner's test	-ve	-ve
Glycosides	Keller – Killiani test	-ve	-ve
Steroids	Salkowski test	-ve	+ve
	Libbermans Burchad	-ve	+ve
Flavonoids	Lead acetate	+ve	-ve
	NaOH solution	+ve	-ve
Tannins	5% FeCl ₃ solution test	+ve	-ve
Terpenoids	Libbermans Burchad	-ve	+ve
Amino acids		-ve	-ve
Starch		+ve	+ve

(+) = Present; (-) = Absent.



Fig. 2: Chromatogram of TLC of ethanolic extract of leaves of *Nyctanthes arbor-tristis* Linn.

TLC plate showing the 6 spots with different colour with different R_f value in iodine chamber in solvent system (Chloroform: Benzene: formic acid) in a ratio of 50:40:10.

Detecting Reagents: Iodine vapour

The six spots with different R_f value have been obtained in TLC of ethanolic extract of leaves of *Nyctanthes arbor-tristis* Linn.

Table 2: List of other solvent system used in T.L.C

S. No.	Solvent System	Solvent ratio	No. of Spots
1	Benzene	100	2
2	Ethyl acetate	100	2
3	Chloroform	100	1
4	Benzene:Ethyl acetate:Formic acid	70:20:10	2
5	Benzene:Ethyl acetate:Formic acid	65:25:10	3
6	Benzene:Ethyl acetate:Formic acid	60:30:10	1
7	Benzene:Ethyl acetate:Formic acid	65:20:15	4
8	Chloroform:Methnol:Formic acid	70:15:15	5
9	Chloroform:Methnol:Formic acid	70:20:10	3
10	Chloroform:Benzene :Formic acid	60:40:few drop	3
11	Hexane : ethyl acetate : Formic acid	60:40: few drops	2
12	Cyclohexane : ethyl acetate	8.5:1.5	4

Table 3: TLC of ethanolic extract of leaves of *Nyctanthes arbor-tristis* Linn.

Extract	Solvent System	No. of Spots	Colour of Spots	R _f value
Ethanolic Extract	Chloroform: Benzene: Formic acid (50: 40: 10)	6	Brown Green Dark Yellow Violet Brown Yellow	0.47 0.57 0.65 0.71 0.80 0.85

The best solvent system for TLC of leaves of *Nyctanthes arbor-tristis* Linn. ethanolic extract is Chloroform: Benzene: Formic acid (50:40:10). TLC of leaves of *Nyctanthes arbor-tristis* Linn. ethanolic extract shows the presence of six compounds with different R_f values in different colour using the iodine vapour as detecting reagent that suggests the presence of six compounds in the extract.

Conclusion

Phytochemical tests were performed on plant extracts to determine the presence of several chemical elements such as Glycoside, Terpenoid, flavonoids, tannins, and other compounds that are responsible for the biological activity of the plant extract. Chemical tests are conducted in an ethanolic extract of *Nyctanthes arbor-tristis* Linn. leaves to detect the presence of several chemical ingredients, and the results are reported in Table 1.

Chloroform was shown to be the most effective solvent system for TLC of leaves of *Nyctanthes arbor-tristis* Linn. ethanolic extract: Benzene: Formic acid is a chemical compound (50:40:10). It was discovered that the ethanolic extract of the leaves of *Nyctanthes arbor-tristis* Linn. exhibited the presence of six compounds with different R_f values in different colours, which was detected using iodine vapour as the detection reagent, indicating the presence of six different compounds in the extract.

These discoveries provided solid evidence that the plant's leaves were from the *Nyctanthes arbor-tristis* Linn. species, which was the source of the plant. Indications for its usage as a medicinal plant include the fact that it is a potent source of a number of chemicals that have therapeutic significance. This can be investigated in greater depth for the purpose of isolating and discovering active biochemical substances that have the potential to be used therapeutically.

Acknowledgement: Nil

Conflicts Of Interest: Nil

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