

Original Research Article

Phytochemical analysis and HPTLC finger printing of *Nyctanthes Arbor-Tristis* Linn. Leaf

ABSTRACT

Herbal medicine also known as botanical medicine refers to using plants seeds, flowers, roots for medicinal purpose. *Nyctanthes arbor-tristis* Linn is a large shrub or small tree, found throughout in India as well as chitrakoot region. It is commonly known as Harshingar, night jasmine, and parijat. It is used for its antibacterial, anthelmintic, anti-inflammatory, hepato-protective, immune potential, antipyretic, antioxidant and antifungal activity. The present investigation deals with preliminary phytochemical screening and High Performance Thin Layer Chromatography (HPTLC) fingerprints profile of Parijat –leaf. For Phytochemical screening two various extracts (ethanolic extract and aqueous extract) of leaf were used and found the Alkaloids, Resins Saponins, Tannins, Terpinoids and Cardiac glycoside present. HPTLC finger printing were carried out on Toluene: Ethyl acetate (7:3); as a mobile phase. The plate were developed over a distance of 9 cm in a saturated development Twin chamber through chamber 10X10 cm with SS lid and visualized under at 254nm, 366nm, under ultraviolet before and after derivatization. After spraying with 5% methanolic sulphuric acid followed by heating at 105⁰C for 5-10 minutes. R_f value were noted with colour before and after derivatization. R_f values are before derivatization at 366nm 0.05, 0.27, 0.31, 0.37, 0.44, 0.51, 0.69, 0.85, 0.92, 0.96 (all red); under ultraviolet: 0.36, 0.41, 0.50, 0.55, 0.95(light yellow). After derivatization at 366nm 0.09 (faint white), 0.31, 0.38, 0.49 (orange), 0.58 (yellow), 0.54, 0.84 (both violet), 0.90 (light pink), 0.96(white); under ultraviolet 0.36, 0.51 (both light brown), 0.58 (light black), 0.98 (brown).

Keywords: Antifungal, phytochemical, antioxidant, derivatization and antroquinone

INTRODUCTION

Herbalism has a long tradition of use of outside of conventional medicine. It is becoming more a main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease worldwide. Herbal medicine also known as phytomedicine-refers to using plants seeds,

flowers, roots for medicinal purpose. Herbal medicine are also refers sometimes botanical medicine.

Nyctanthes arbor-tristis Linn is commonly known as Harshingar, night jasmine, and parijat, it belongs to Nyctanthaceae family; it is a large shrub or small tree, found throughout in India as well as Chitrakoot region. It is also planted in Indian gardens for ornamental purpose due to its highly fragrant flowers. It is a shrub or small tree up to 10 m in height with gray to greenish rough bark with stiff whitish hairs. Leaves are opposite, ovate, acute or acuminate, entire or with few large distant teeth; short bulbous hairs rounded or slightly cuneate (Fig. 1.1). Flowers are small, delightful fragrant, sessile, slender, and hairy; corolla glabrous, orange colored and lobes are white. Fruits are a capsules of 1-2 m in diameter, long and broad, compressed, 2 celled separating into 2 flat one seeded carpels, reticular veined and glabrous (Saxena, 2002; Ratnasooriya, 2005). Different parts of *Nyctanthes arbor-tristis* are known to own for the treatment of various ailments by tribal people of India especially Orissa and Bihar along with its use in Ayurveda, Siddha and Unani systems of medicines (Suresh, 2001; Sasmal, 2007). The barks of Harsingar intended for expectorant, anorexia, liver disorder, piles, worm infestation, blood disorder, oliguria, skin diseases fever and snake bite. The leaves of this plant are also used against arthritis and malarial fever (Yadav *et al.*, 2013).

Phytochemistry is the study of phyto-co-nutrient produced in plants, describing the isolation, purification, identification, of the large number of secondary metabolic compounds found in plants. Effect of extracted plant phytochemicals depends on: the natural condition of the plant material, its origin and degree of processing (Islam, 2012). 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 (Levu *et al.*, 2013). High Performance Thin Layer Chromatography (HPTLC) is a sophisticated and automated form of Thin Layer Chromatography (TLC) techniques. The method is used for separation of the components present in mixture both quantitatively as well as qualitatively.

Derivatization: *In situ* derivatization possibility is a strong point of HPTLC. Chemical reactions are possible in situ on the plate before or after chromatography both the possibility possibilities have their advantages. However, the decision depends on sample matrix level of detection and interference present in the sample. Post chromatography derivatization is a

more popular technique for which several hundred references are available in literature as compared to a few for pre-chromatographic derivatization. The results are unique and specific when derivatization before chromatographic development has been recommended. (Ahirwar, 2012)

MATERIALS AND METHODS

Collection of the plant

The plant material was collected from bagdraghati region in chitrakoot (Satna district) and transported to the laboratory. The plant was identified by the Prof. G. P. Richhariya, Prof. of Botany, Govt. College, Amarpatan, Satna (M.P.). A specimen was preserved in the college herbarium (PGCS/Rec./2015). Plant samples ~~was~~ were washed, dried, grinded to powder form and stored for further analysis.

Sample preparation: -Taken 2 gm of sample powder transferred into 250 ml conical flask then added 100 ml ethanol for ethanolic extract and water for water extract then kept on ~~rotatory~~ rotatory flask shaker for 6hr. after shaker leave for 18hr for maceration procedure after maceration filter through whatt's-man filter paper then the filtrate is used for phytochemical analysis.

Phytochemical Screening of Alkaloids:-

A. Dragandroff's Test – Dissolved a few ml of alcohol or aq. extract of drug in 5ml of distilled water then added 1ml HCL until an acid reaction occurs then added 1ml of Dragandriff's reagent an orange or orange red ppt. is produced immediately.

B. Wagner's Test –Acidify 1ml of alcoholic drug extract ~~of drug~~ with 1.5% w/v of HCl and few drops of wager's reagent, a yellow or brown precipitate was formed.

C. Mayer's Test-~~added~~ few drops of Mayer's reagent to 1ml of acidic aq. Extract of the drug white or pole yellow is formed.

1. Phytochemical Screening Carbohydrates:-

A. Anthrone's Test – 2 ml of Anthrone's test solution add 0.5ml of aq. Extract of drug. A green or blue colour indicates the presence of Carbohydrates.

B. Fehling's Test: - 2 ml of aq. Extract of drug add 1ml of mix. Of equal parts of Fehling's solution 'A' and 'B' and boil the contents of the test tube for few minutes a Red or Pink-red precipitate is formed.

C. Molisch's Test: -In a test tube containing 2 ml of aq. Extract of drug added 2 drops of freshly prepared 20% alcoholic solution of α -naphthol and mix pour 2ml H₂SO₄concentrated ~~H₂SO₄—SO~~ [RGG1] as to form a layer below the mixture.

Carbohydrate if present Red-Violet ring, which disappear on the addition of an excess alkali solution.

2. Phytochemical Screening Flavonoids :-

In test tube containing 0.5ml of alcoholic extract of drug added 5-10 drops of diluted HCl followed by small pieces of 'Mg' in the presence of Flavonoids Pink, Reddish -pink or brown colour was produced

3. Phytochemical Screening Proteins :-

A. Bieuret's Test: - 1ml of hot aq. Extract of drug added 5-8 drops of 10% w/v NaOH solution followed by 1 to 2 drop of 3% w/v CuSO₄ solution Red or Violet colour was developed.

B. Millon's Test: -Dissolved small quantity of aq. Extract of drug in 1ml of distilled water and added 5-6 drops of millon's reagent a white precipitate is formed which turns Red on heating.

4. Phytochemical Screening Resins:-

Took Dissolved water extracts in 1ml acetone and mixed the solution into 5ml distilled water, turbidity indicates the presence of Resins.

5. Phytochemical Screening Saponins: - 5ml of an aq. extract of drug added drop by drop into the solution of sodium bicarbonate (NaHCO₃) shake it vigorously and left for few minutes Honey-Comb like structure is formed.

6. Phytochemical Screening Tannins: -1-2 ml of extract of water drug added few drops of 5% FeCl₃ solution a greenish colour were developed that indicates the presence of tannin. White Brown colour tannins.

7. Phytochemical Screening Starch: - Dissolved 0.015g of iodine and 0.075g of KI in 5 ml of distilled water and add 2-3ml of an aq. extract of drug a blue colour is produce.

8. Phytochemical Screening Steroids: - 2 ml of acidic anhydride was added to 0.5ml ethanolic extract each sample with 2 ml H₂SO₄ the colour changed from Violet to Blue or Green in some sample indicating the presence of steroids.

9. Phytochemical Screening Terpenoids:-

Salkowski Test: - 5 ml of each extract was-were mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show the presence of terpinoids.

10. Phytochemical Screening Cardiac glycoside:-

Keller-Killani Test: - 5 ml of each extract ~~was-were~~ treated with 2 ml of glacial acetic acid containing one drop of ferric chloride ~~solution~~^[RGG3] this was underplayed with 1ml of concentrate H₂SO₄. A brown ring of the interface indicates a deoxysugar characteristics cardinolides. A violet ring may appear below the brown ring while in the acidic acid layer, a greenish ring may from just gradually throughout thin layer.

11. Phytochemical Screening Anthroquinones:-

Borntrger's Test: - 0.5ml of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added a pink, red or violet ~~colouretion~~^{coloration} in the ammonical phase indicated the presence of Antroquinone.

High Performance Thin Layer Chromatography (HPTLC)

HPTLC were carried out on Silica gel 60 F₂₅₄ pre-coated Aluminiumplate's 0.2 mm thickness, Merck, Mumbai. An Applicator from Camag Linomat-5 (Camag Switzerland: 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints. The mobile phase used for leaf ~~was~~ Toluene:-Ethyl acetate (7:3); ~~Volume-volume~~ of test sample applied : 8 μ l. The plate ware developed over a distance of 9 cm in a saturated development chamber (Twin ~~through~~ chamber 10X10 cm with SS lid and visualized under at 366nm, under ultraviolet before and after derivatization. After spraying with 5% *methanolic sulphuric* acid followed by heating at 105⁰C for 5-10 minutes.

Table 1: Chromatographic conditions for MEL

Extract	Stationary phase	Solvent system	Detection
MEL	On Silica gel 60 F ₂₅₄ pre-coated Aluminium plates	Toluene : Ethyl acetate (7:3)	spraying with 5% <i>methanolic</i> H ₂ S ~~O~~ ₄ after drying heated at 105 ⁰ C for 5-10 minutes

MEL: Methanolic extract of Leaf

RESULTS AND DISCUSSION

The present investigation deals with the study of ~~a~~ phytochemical screening and HPTLC fingerprints profile of *Nictanthus abour-tristis* Linn. ~~Leaf for~~ Phyto-chemical screening two various extracts (ethanolic extract and aqueous extract) of leaf were used and found the ~~[RGG4]~~ Alkaloids, Resins Saponins, Tannins, Terpinoids and Cardiac glycoside present.

Whereas carbohydrate flavonoids, protein, starch, anthraquinone are absent (table: 2) this is in agreement with the report of (Suresh and Arunachalam 2012). The previous researchers—also reported the presence of alkaloids, carbohydrate, glycosides, phytosterols, fixed oil, tannins, flavonoids, proteins and amino acids, gums and mucilage (WHO, 2002; Willis, 1973; Chase And Pratt, 1949), contain tannic acid, methyl salicylate, amorphous glucosides, mannitol, resin, ascorbic acid, carotene, and traces of a volatile oil (Shinde *et al.*, 2014). HPTLC R_f value was noted with colour before and after derivatization, R_f values are Before derivatization at 366nm 0.05, 0.27, 0.31, 0.37, 0.44, 0.51, 0.69, 0.85, 0.92, 0.96 (all red) (table: 3); under ultraviolet: 0.36, 0.41, 0.50, 0.55, 0.95 (light yellow) (table: 4). After derivatization at 366nm 0.09 (faint white), 0.31, 0.38, and 0.49 (orange), 0.5 (yellow), 0.54, 0.84 (both violet), 0.90 (light pink), 0.96 (white) (table 5); under ultraviolet 0.36, 0.51 (both light brown), 0.58 (light black), 0.98 (brown) (table: 6).

Table 2: Phyto-chemical screening of *Nictanthus abour-tristis* Linn

S. No.	Phyto-constituents	Observation	Results
1.	Alkaloids A. Dragendorff's test B. Wagner's test C. Mayer's test	Orange colour seen Brown colour produced Brown colour produced	+ + +
2.	Carbohydrate A. Anthrone's test B. Fehling's test C. Molisch's test	Chocolaty colour seen Blue colour seen Brown ring not appeared	— — —
3.	Flavonoids	Yellowish color appear	—
4.	Protein (Bieuret's test)	brown colour appear	—
5.	Resins	Turbidity appear	+
6.	Saponins	Honey comb like structure found	+
7.	Tannins	Greenish colour appeared	+
8.	Starch	brown colour seen	—
9.	Steroids	Brown ring appeared	+
10.	Terpinoids	Radish brown coloration between interface was seen	+
11.	Cardiac glycoside	Reddish brown ring and greenish	+

		ring seen	
12.	Antraquinone	White colour seen	—

(+): Present, (-): Absent

HPTLC Analysis:-

Table 3: *R_f* value of the MEL Before Derivatization 366nm

<i>R_f</i> value (Colour)	Before Derivatization 366nm	
	Spot –A	Spot –B
<i>R_{f1}</i> (Red)	0.05	0.05
<i>R_{f2}</i> (Red)	0.27	0.27
<i>R_{f3}</i> (Red)	0.31	0.31
<i>R_{f4}</i> (Red)	0.37	0.37
<i>R_{f5}</i> (Red)	0.44	0.44
<i>R_{f6}</i> (Red)	0.51	0.51
<i>R_{f7}</i> (Red)	0.69	0.69
<i>R_{f8}</i> (Red)	0.85	0.85
<i>R_{f9}</i> (Red)	0.92	0.92
<i>R_{f10}</i> (Red)	0.96	0.96

Table 4: *R_f* value of the MEL Before Derivatization in ultraviolet

<i>R_f</i> value (Colour)	Before Derivatization ultraviolet	
	Spot –A	Spot –B
<i>R_{f1}</i> (Light yellow)	0.36	0.36
<i>R_{f2}</i> (Light yellow)	0.41	0.41
<i>R_{f3}</i> (Light yellow)	0.50	0.50
<i>R_{f4}</i> (Light yellow)	0.55	0.55
<i>R_{f5}</i> (Light yellow)	0.95	0.95

Table 5: *R_f* value of the MEL After Derivatization 366nm

<i>R_f</i> value (Colour)	After Derivatization 366nm	
	Spot –A	Spot –B
<i>R_{f1}</i> (Faint white)	0.09	0.09
<i>R_{f2}</i> (Orange)	0.31	0.31
<i>R_{f3}</i> (Orange)	0.38	0.38

R _{f4} (Orange)	0.49	0.49
R _{f5} (Yellow)	0.58	0.58
R _{f6} (Violet)	0.54	0.54
R _{f7} (Violet)	0.84	0.84
R _{f8} (Light pink)	0.90	0.90
R _{f9} (White)	0.96	0.96

Table 6: R_f
MEL After

value of the

Derivatization in ultraviolet

R_f value (Colour)	After Derivatization visible light	
	Spot –A	Spot –B
R _{f1} (Light brown)	0.36	0.36
R _{f2} (Light brown)	0.51	0.51
R _{f3} (Light black)	0.58	0.58
R _{f4} (Light black)	0.65	0.65
R _{f5} (Brown)	0.98	0.98



Fig. 1.1: Shows morphological character of *Nyctanthes arbor-tristis* Leaves

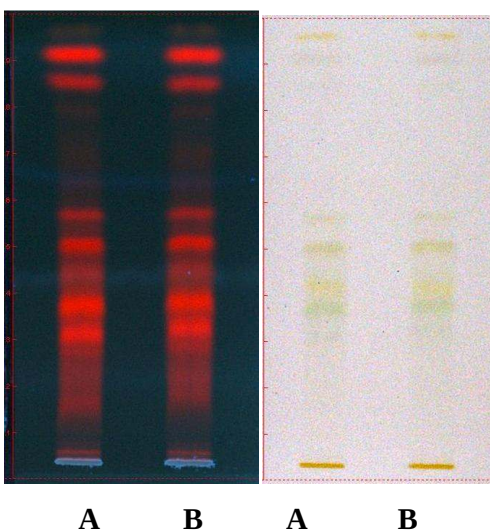


Fig. 2-3: HPTLC profile MEL before Derivatization under 366nm & ultraviolet

Track 1: spot A, Track 2: spot B.

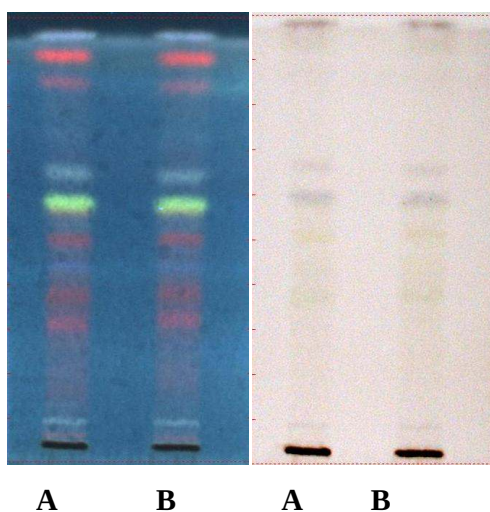


Fig. 4-5: HPTLC profile of MEL after Derivatization under 366nm & ultraviolet Track 1: spot A, Track 2: spot B.

CONCLUSION

Phytochemical screening and HPTLC fingerprinting profiles are distinctive and characteristics both of them together they may be used for quality evaluation and maintaining their quality, purity and efficacy. In the present study Preliminary-preliminary phytochemical investigations were carried out for the identification of chemical constituent present in *Nictanthus abour-tristis* Linn. HPTLC profiles are very important for monitoring the quality

of the *Nictanthus abour-tristis* Linn. Leaf for establishing whether the required ingredients are present in them, it may be used for standardization and developing a protocol for drug preparation.

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