

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, hepatoprotective, immune potential, antipyretic, antioxidant and antifungal activity. The present investigation deals with preliminary phytochemical screening and HPTLC fingerprints profile of Parijat –leaf. For Phyto-chemical screening two various extracts (ethanolic extract and aqueous extract) of leaf were used and found the Alkaloids, Resins, Saponins, Tannins, Terpenoids and Cardiac glycoside present. HPTLC were carried out on Toluene: Ethyl acetate (7:3); as a mobile phase. The plate was developed over a distance of 9 cm in a saturated development chamber. Twin 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 105°C for 5-10 minutes. R_f values 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 anthraquinone

INTRODUCTION

Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream 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,

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

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36 *Nyctanthes arbor-tristis* Linn is commonly known as Harshingar, night jasmine, and parijat, it
37 belongs to Nyctanthaceae family; it is a large shrub or small tree, found throughout in India as
38 well as Chitrakoot region. *Nyctanthes arbor-tristis* Linn is used for its antibacterial,
39 anthelmintic, anti-inflammatory, hepatoprotective, immunopotential, antipyretic, antioxidant
40 and anti fungal activity. The barks of Harsingar intended for expectorant, anorexia, liver
41 disorder, piles, worm infestation, blood disorder, oliguria, skin diseases fever and snake bite.
42 The leaves of this plant used against arthritis and malarial fever (yadav *et al.*, 2013).

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44 Phytochemistry is the study of phytoco-nutrient produced in plants, describing the isolation,
45 purification, identification, of the large number of secondary metabolic compounds found in
46 plants. Effect of extracted plant phytochemicals depends on: the natural condition of the plant
47 material, its origin and degree of processing (Islam, 2012). Phytochemicals are non-nutritive
48 plant chemicals that have protective or disease preventive properties. The qualitative and
49 quantitative estimation of the phytochemical constituents of a medicinal plant is considered to
50 be an important step in medicinal plant research (Levuet *et al.*, 2013). High performance thin
51 layer chromatography (HPTLC) is a sophisticated and automated form of Thin Layer
52 Chromatography (TLC) techniques. The method is used for separation of the components
53 present in mixture both quantitatively as well as qualitatively.

54 **Derivatization:** In situ derivatization possibility is a strong point of HPTLC. Chemical
55 reactions are possible in situ on the plate before or after chromatography both the possibility
56 have their advantages. However the decision depends on sample matrix level of detection and
57 interference present. Post chromatography derivatization is more popular technique for which
58 several hundred references are available in literature as compared to a few for pre-
59 chromatographic derivatization. The results are unique and specific when derivatization
60 before chromatographic development has been recommended. (Ahirwar, 2012)

61 MATERIALS AND METHODS

62 Collection of the plant

63 The plant material was collected from bagdraghati region in chitrakoot (Satna district) and
64 transported to the laboratory. The plant was identified by the supervisor. A specimen was

65 preserved in the college herbarium (PGCS/Rec./2015). Plant sample was washed, dried,
66 grinded to powder form and stored for further analysis.

67 **Sample preparation:** -Taken 2gm of sample powder transferred into 250ml conical flask
68 then added 100 ml ethanol for ethanolic extract and water for water extract then kept on
69 rotatory flask shaker for 6hr. after shaker leave for 18hr for maceration procedure after
70 maceration filter through whattman filter paper then the filtrate is used for phytochemical
71 analysis.

72 **Phytochemical Screening of Alkaloids:-**

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

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

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

80 **1. Phytochemical Screening Carbohydrates:-**

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

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

86 **C. Molisch's Test:** -In a test tube containing 2ml of aq. Extract of drug added 2 drops of
87 freshly prepared 20% alcoholic solution of α -naphthol and mix pour 2ml concentrate
88 H_2SO_4 so as to form a layer below the mixture. Carbohydrate if present Red-Violet
89 ring, which disappear on the addition of an excess alkali solution.

90 **2. Phytochemical Screening Flavonoids :-**

91 In test tube containing 0.5ml of alcoholic extract of drug add 5-10 drops of dilute HCl
92 followed by small pieces of 'Mg' in the presence of Flavonoids Pink, Reddish -pink or
93 brown colour is produce

94 **3. Phytochemical Screening Proteins :-**

95 **A. Bieuret's Test:** - 1ml of hot aq. Extract of drug add 5-8 drops of 10% w/v NaOH
96 solution followed by 1 to 2 drop of 3% w/v $CuSO_4$ solution a Red or Violet colour is
97 obtained.

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

101 **4. Phytochemical Screening Resins:-**

102 Dissolve the water extracts in 1ml acetone and pours the solution in to 5ml distilled water
103 turbidity indicates the presence of Resins.

104 **5. Phytochemical Screening Saponins:-**in test tube containing about 5ml of an aq. Extract
105 of drug add drop of solution of sodium bicarbonate (NaHCO_3) shake it vigorously and
106 left for few minutes Honey-Comb like structure is formed

107 **6. Phytochemical Screening Tannins:** -1-2 ml of extract of water drug added few drops of
108 5% FeCl_3 solution a greenish colour developed indicates the presence of tannin. White
109 Brown colour tannins.

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

112 **8. Phytochemical Screening Steroids:** -2ml of acidic anhydride was added to 0.5ml
113 ethanolic extract each sample with 2ml H_2SO_4 the colour changed from Violet to Blue or
114 Green in some sample indicating the presence of steroids.

115 **9. Phytochemical Screening Terpenoids:-**

116 **Salkowski Test:** - 5ml of each extract was mixed in 2ml of chloroform, and
117 concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown
118 coloration of the interface was formed to show the presence of terpenoids.

119 **10. Phytochemical Screening Cardiac glycoside:-**

120 **Keller-Killani Test:** - 5ml of each extract was treated with 2ml of glacial acetic acid
121 containing one drop of ferric chloride solution this was underplayed with 1ml of
122 concentrate H_2SO_4 . A brown ring of the interface indicates a deoxysugar characteristics
123 cardinolides. A violet ring may appear below the brown ring while in the acidic acid
124 layer, a greenish ring may form just gradually throughout thin layer.

125 **11. Phytochemical Screening Anthroquinones:-**

126 **Borntrger's Test:** - 0.5ml of the plant extract was shaken with benzene layer separated
127 and half of its own volume of 10% ammonia solution added a pink, red or violet
128 colouration in the ammonical phase indicated the presence of Anthroquinone.

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High Performance Thin layer chromatography

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 Toluene : Ethyl acetate (7:3); Volume of test sample applied : 8µ. The plate were 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 SO 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 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 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). 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).

157 **Table: 2**Phyto-chemical screening of *Nictanthusabour-tristis* Linn. :-

S. No.	Phyto-constituents	Observation	Results of
1.	Alkeloids A. Dragendroff's test B. Wagner's test C. Mayer's test	Orange colour seen Brown colour produced Brown colourproduced	+ + +
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	—

158 (+): Present, (-): Absent

159 HPTLC Analysis:-

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161 **Table: 3 - Rfvalueof the MEL Before Derivatization366nm :-**

Rfvalue (Colour)	Before Derivatization 366nm	
	Spot –A	Spot –B
R _{f1} (Red)	0.05	0.05
R _{f2} (Red)	0.27	0.27
R _{f3} (Red)	0.31	0.31
R _{f4} (Red)	0.37	0.37

Rf_5 (Red)	0.44	0.44
Rf_6 (Red)	0.51	0.51
Rf_7 (Red)	0.69	0.69
Rf_8 (Red)	0.85	0.85
Rf_9 (Red)	0.92	0.92
Rf_{10} (Red)	0.96	0.96

Table: 4 - Rf value of the MEL Before Derivatization in ultraviolet:-

Rfvalue (Colour)	Before Derivatization ultraviolet	
	Spot –A	Spot –B
Rf_1 (Light yellow)	0.36	0.36
Rf_2 (Light yellow)	0.41	0.41
Rf_3 (Light yellow)	0.50	0.50
Rf_4 (Light yellow)	0.55	0.55
Rf_5 (Light yellow)	0.95	0.95

Table: 5 - Rf value of the MEL After Derivatization366nm :-

Rfvalue (Colour)	After Derivatization 366nm	
	Spot –A	Spot –B
Rf_1 (Faint white)	0.09	0.09
Rf_2 (Orange)	0.31	0.31
Rf_3 (Orange)	0.38	0.38
Rf_4 (Orange)	0.49	0.49
Rf_5 (Yellow)	0.58	0.58
Rf_6 (Violet)	0.54	0.54
Rf_7 (Violet)	0.84	0.84
Rf_8 (Light pink)	0.90	0.90
Rf_9 (White)	0.96	0.96

**Table: 6 -
the MEL**

**Rf value of
After**

Derivatization in ultraviolet:-

Rfvalue (Colour)	After Derivatization visible light	
	Spot –A	Spot –B
Rf_1 (Light brown)	0.36	0.36
Rf_2 (Light brown)	0.51	0.51

Rf_3 (Light black)	0.58	0.58
Rf_4 (Light black)	0.65	0.65
Rf_5 (Brown)	0.98	0.98

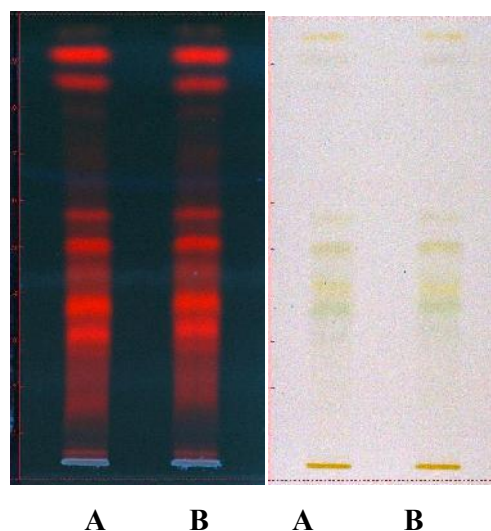


Fig. 1-2: HPTLC profile MEL Before Derivatization under 366nm & ultraviolet
Track 1: spot A,
Track 2: spot B.

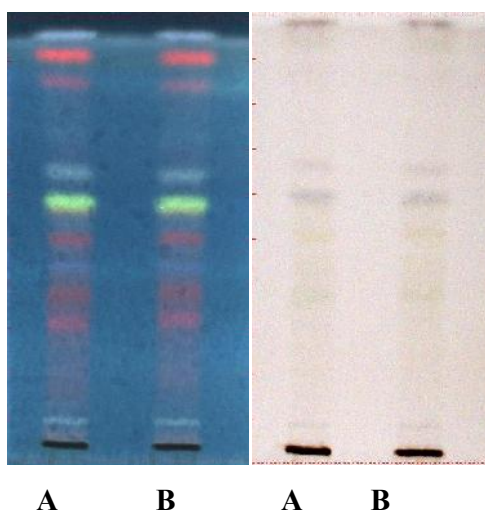


Fig. 3-4: HPTLC profile of MEL After Derivatization under 366nm & ultraviolet
Track 1: spot A,
Track 2: spot B.

CONCLUSION

Present studies, it can be calculated that the preliminary phytochemical investigation for the identification of chemical constituent present in *Nictanthus abour-tristis* Linn and the HPTLC

profiles are very important for monitoring the quality of the *Nictanthusabour-tristis*Linn. Leaffor establishing whether the required ingredients are present in them,It is also helpful for standardization and developing a protocol for drug preparation.Phytochemical screening and HPTLC fingerprinting profiles are characteristics together may be used for qualityevaluation and maintaining their quality, purity andefficacy.

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