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Determination of Kaempferol by HPTLC Method from *Nyctanthes Arbortristis* Linn Flower Extract

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ABSTRACT

The evaluation of the composition of the phytoconstituents of the medicinally important plant Nyctanthes arbortristis Linn. (Nyctanthaceae) was done with the help of HPTLC fingerprint sequence of the plant. The preliminary qualitative phytochemical screening and the HPTLC fingerprint analysis were carried out. The developing solvents were toluene: chloroform:ethanol (4:4:2,v/v/v) was employed. The phytochemical screening showed the presence of unknown phenolic and kaempferol phyto compounds. The HPTLC fingerprinting of the extracts showed several peaks with different R_f values. The ethanol extractof flowers sample showed 13 and 17 peaks of 5.0 mg ml^{-1} sample solution in 10 µL at 200 and 254 nm. The HPTLC fingerprint profile is used in differentiation of the species from the adulterant and act as biochemical markers for this medicinally important plant of Nyctanthes arbortristis. It may be used to pharma industry and plant systematic studies.

Graphical Abstract:

Flowers of Nyctanthes arbortristis Linn

Keywords: *Nyctanthes arbortristis*, HPTLC, Kaempferol, Phenolics.

INTRODUCTION

Traditional medicine (TM) is due a revival. For millennia, people around the world have healed the sick with herbal or animal-derived remedies, handed down through generations. In Africa and Asia, 80 per cent of the population medical students believe that Western medicine would benefit by integrating traditional or still uses traditional remedies rather than modern medicine for primary healthcare. And in developed nations, TM is rapidly gaining appeal. Estimates suggest up to 80 per cent of the population has tried a therapy such as acupuncture or homeopathy. And a survey conducted earlier this year found that 74 per cent of US alternative therapies and practices. Phytochemical analysis of plants were used in folklore has yielded a number of compounds with various pharmacological activities. Plants which are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids etc., have been found to have several biological properties. So the use of and search for drugs and dietary supplement derived from plants have increased in recent years [1]. Standardization of the plant material is need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physico-chemical characters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and its formulations. The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards [2, 3]. HPTLC is a simple, rapid and accurate method for analyzing plant material [4]. HPTLC fingerprint has better resolution and estimation of active constituents is done with reasonable accuracy in a shorter time. The HPTLC method can be used for phytochemical profiling of plants and quantification of compounds present in plants, with increasing demand for herbal products as medicines and cosmetics there is an urgent need for standardization of plant products [5]. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to ITM (Indian Traditional Medicine) and TCHM (Chinese traditional herbal medicine). The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs and to preserve such "database" for further multifaceal sustainable studies. HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug [6].

Nyctanthes arbortristis 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. 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 slight cuneate (Fig. 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 [7,8]. Different parts of Nyctanthes arbortristis are known to own for 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 [9,10]. The barks of Harshingar intended for expectorant, anorexia, liver disorder, piles, worm infestation, blood disorder, oliguria, skin diseases fever and snake bite. The leaves of this plant used against arthritis and malarial fever [11]. 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.

MATERIALS AND METHODS

Collection and Identification of Plant Material: The fresh flowers (Fig. 1) were collected during the month of October 2013 from different parts of Kalyan, Maharashtra, India. The taxonomic identity of the plant was confirmed by Dr. S. Sharma, at Botany Department,M.D. College, Mumbai, Maharashtra. The

flowers were washed and air dried under shade. The dried sample was powdered and used for further studies.

Fig 1. Flowers of Nyctanthes arbortristis Linn

Thin Layer Chromatography of Ethanolic extract: Ethanolic extract of Nyctanthes arbortristis flower sample was subjected to thin layer chromatographic studies, to find out the probable number of compounds present in them.

Extraction and Test solution preparation: The dried and crushed flowers (1 Kg) were extracted with ethanol in Soxhlet apparatus for 24 h. Then the resulting sample was cooled, filtered and concentrated with a rotary evaporator at low temperature (40-50^{0}C). After evaporation of solvent in vacuum, dark brown gummy mass (40 g) was obtained. About 5 mg of this mass was used for the HPTLC analysis and the remaining part is refrigerated for further study. This 5 mg of gummy mass was dissolved in 1 mL methanol and centrifuged at 3000 rpm for 5 min. This solution was used as test solution for HPTLC analysis [12].

Sample loading, Spot development, Photo documentation and Scanning: 1.5µL of the above test solution and 2µL of standard solutions were loaded as 6mm band length in the 10 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapour) with respective mobile phase (Flavonoid) and the plate was developed in the respective mobile phase up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo documentation chamber (CAMAG Visualizer!150503) and captured the images at White light, UV 254nm and UV366nm. The developed plate was sprayed with respective spray reagent (Flavonoid) and dried at 100°C in Hot air oven. The plate was photo documented at Day light and UV 366nm using Photo documentation (CAMAG Visualizer!150503) chamber. Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254nm. The Peak table, Peak display and Peak Densitogram were noted.

HPTLC Study: HPTLC precoated, silica gel 60 F254 (Merck, Germany) plates were used for application of sample. A small quantity of extract was dissolved in methanol and sample was applied in precoated plate with the help of Linomat V applicator. Solvent system optimized for TLC study was chosen for HPTLC study. The details of HPTLC were as follows

Plate	Aluminium plate precoated with silica gel 60F254
Thickness	2µm
Plate size	10 × 10 cms
Sample application	10 µ1
Solvent system	Toluene: Chloroform:Ethanol (4:4:2)
Detection	U.V. (254,366 nm)
Instrument	CAMAG TLC Scanner 3 & LINOMAT- V

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the ethanolic extract revealed the presence of chemical constituents like Steroids, Tannins, Flavonoids, Carbohydrates, Saponins, Amino acids and alkaloids which was confirmed by performing TLC separation technique and different spraying reagents (the data was not given). The use of medicinal plants as Herbal Drugs, extracts have significantly increased throughout the world in the recent decades. According to WHO 80% of world population uses herbal remedies to cure diseases. Taking into consideration the popularity of the medicinal plants, these traditional drugs should be evaluated in detail for their Pharmacognosy, Phytochemistry and Pharmacology. Such a standardization of traditional medicine is the step to tap the emerging export market for natural drugs. All these medicinally important traditional crude drugs and their extracts should be standardized for its quality, purity and safety by using techniques such as organoleptic, microscopical, physical, chemical, biological evaluation. Pharmacognostic study mainly involves correct identity of crude drug with the help of drug organoleptic (sensory characters), microscopical (histological characters), physical and preliminary chemical evaluation [13]. Present study has given very important information about High Performance Liquid Chromatographic (HPTLC) determination showed presence of flavonoids like kaempferol in the ethanolic extract of Nyctanthes arbortristis. Most of the secondary metabolites found from herbs and spices are commercially important and are being used as pharmaceutically active compounds. Especially, flavonoids and phenolic acids are the key groups of secondary metabolites and bioactive compounds in plants [14]. They are also kind of natural products and antioxidant substances capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer. Secondary metabolites are chemicals produced by plants for defense mechanism; and their functions in growth, photosynthesis, reproduction and other primary processes are not known yet. Secondary chemicals are also important while enumerate the plant uses widely in Asia [15]. The ethanolic extract of Nyctanthes arbortristis contains phenolics, flavonoids and terpenoids which was analyzed by using HPTLC. The peak table, peak display and peak Densitogram were noted. Blue-violet colored zones were detected from the chromatogram after derivatization which confirmed the presence of phenolics (Fig. 3,4). Thus, the presence of flavonoids in the ethanolic extract of the flowers of Nyctanthes arbortristis was confirmed by HPTLC analysis. The extract was run along with the standard flavonoid compound and it was observed that the extract showed the presence of presence of phenolic and flavonoids and it was confirmed from the chromatogram after derivatization. The peak heights of the respective kaempferol standard and phenolic compounds were given in the Figs. 2, 3, 4, 5 and Table 1, 2.

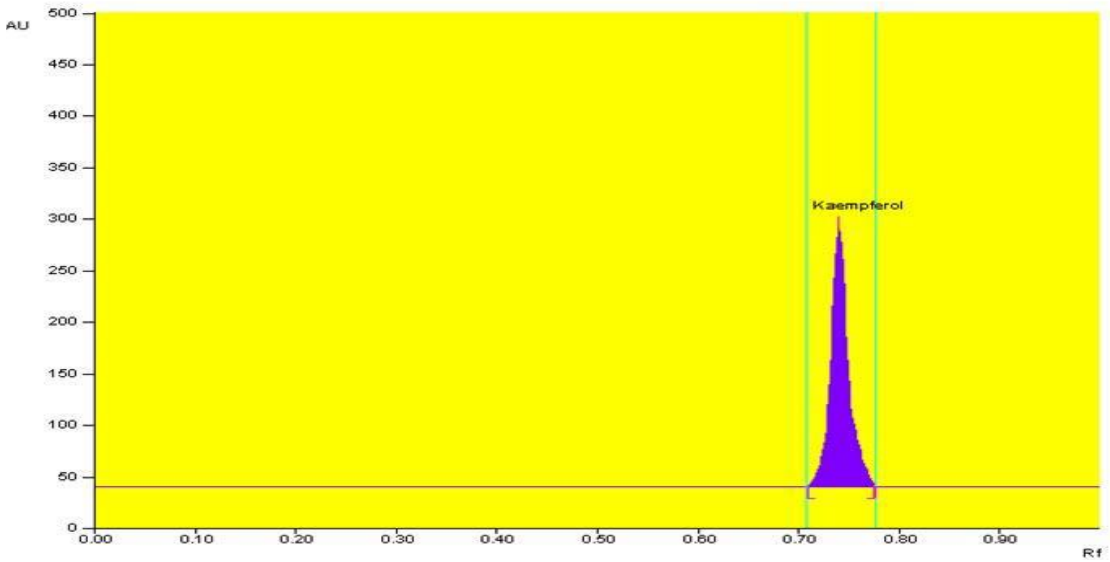

Fig 2. Kaempferol standard peak Densitogram display (254 nm)

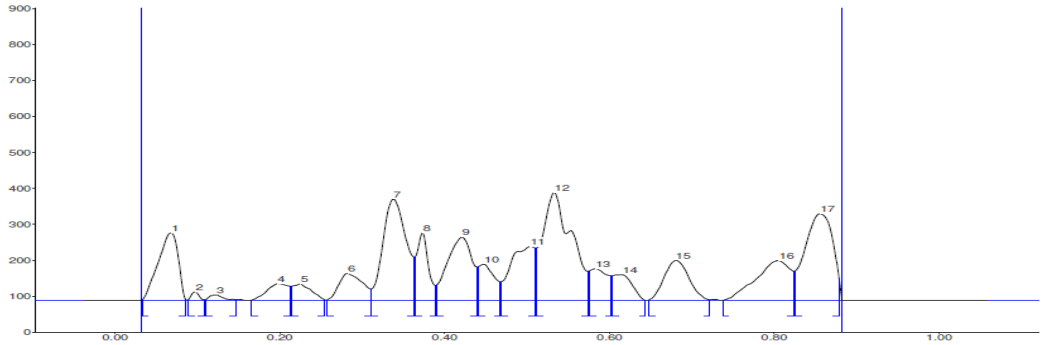

Fig 3. Chromatogram of Ethanol extracts *of N. arbortristis flowers* at 200 nm

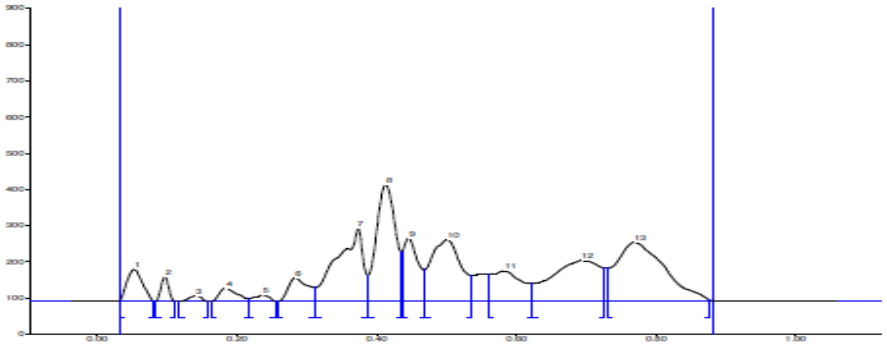

Fig 4. Chromatogram of Ethanol extracts *of N. arbortristis flowers* at 254 nm

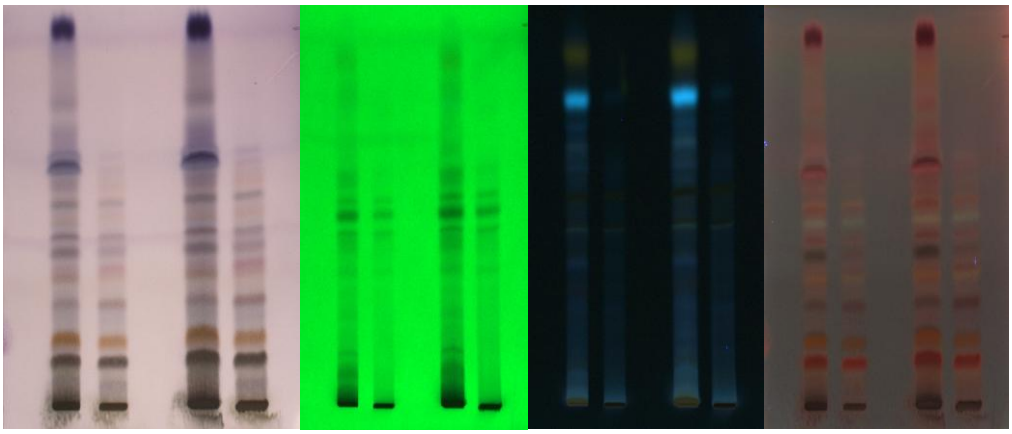

Fig 5. Sample N. arbortristis flowers ethanolic extract sample peak display(Scanned at day light, 254nm, 366nm-plate after derivation, 366 nm –plate before derivation)

Table 1: Peak Value at 200 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area%	Assigned Substance
1	0.03	0.6	0.07	186.3	8.50	0.09	3.2	44289.2	7.36	Unknown
2	0.09	1.0	0.10	22.1	1.01	0.11	0.7	192.1	0.33	Unknown
3	0.11	1.2	0.12	14.5	0.66	0.15	0.8	214.9	0.37	Unknown

4	0.17	0.2	0.20	45.1	2.06	0.21	38.5	1130.2	1.94	Unknown
5	0.21	38.5	0.23	45.2	2.06	0.25	0.8	952.8	1.64	Unknown
6	0.26	0.1	0.28	74.5	3.40	0.31	30.7	2019.0	3.47	Unknown
7	0.31	31.6	0.34	280.7	12.80	0.36	119.3	7501.3	12.88	Unknown
8	0.36	119.8	0.37	185.6	8.46	0.39	40.6	2541.9	4.36	Unknown
9	0.39	41.9	0.42	174.9	7.97	0.44	91.9	4963.0	8.52	Unknown
10	0.44	92.5	0.45	99.8	4.55	0.47	51.1	1833.7	3.15	Unknown
11	0.47	51.2	0.50	149.4	6.81	051	144.5	4101.9	7.04	Unknown
12	0.51	144.6	0.53	298.7	13.62	0.58	80.0	10032.8	17.22	Unknown
13	0.58	81.0	0.58	87.1	3.97	0.60	67.2	1800.5	3.09	Unknown
14	0.60	67.6	0.62	70.8	3.23	0.65	0.1	1533.7	2.63	Unknown
15	0.65	0.2	0.68	110.0	5.02	0.72	0.8	3118.5	5.35	Unknown
16	0.74	0.2	0.81	109.1	4.97	0.83	80.0	4333.1	7.44	Kaempferol
17	0.83	80.7	086	239.7	10.93	0.88	60.3	7689.6	13.20	Unknown

Table 2: Peak Value at 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area%	Assigned Substance
1	0.03	2.8	0.05	89.1	5.82	0.06	0.9	1899.9	3.50	Unknown
2	0.08	0.4	0.10	68.3	4.46	0.11	0.0	714.1	1.31	Unknown
3	0.12	0.4	0.14	16.8	1.10	0.16	0.2	285.6	0.53	Unknown
4	0.16	0.1	0.19	37.5	2.45	0.22	8.9	923.3	1.70	Unknown
5	0.22	9.0	0.24	18.4	1.20	0.26	0.2	384.6	0.71	Unknown
6	0.26	0.6	0.28	65.4	4.27	0.31	38.8	17.87.7	3.29	Unknown
7	0.31	39.0	0.37	201.9	13.18	0.39	73.4	7178.4	13.22	Unknown
8	0.39	73.5	0.41	322.9	21.09	0.44	136.9	8537.3	15.72	Unknown
9	0.44	139.0	0.45	176.1	11.50	0.47	87.7	3642.2	6.71	Unknown
10	0.47	88.7	0.50	171.5	11.20	0.54	72.1	6955.0	12.81	Unknown
11	0.56	75.4	0.58	85.5	5.58	0.62	49.4	3566.3	6.57	Unknown
12	0.62	49.7	0.70	114.0	7.44	0.73	93.5	7503.8	13.82	Unknown
13	0.73	92.3	0.77	163.7	10.69	0.88	4.2	10933.0	20.13	Kaempferol

APPLICATIONS

Kaempferol obtained from the ethanolic extract of Nyctanthes arbor-tristis have the reputation of being anticarcinogenic compound. Isolation of Kaempferol from the flowers of Nyctanthes arbor-tristis can reduces the cost of chemical synthesis of the same.

CONCLUSIONS

A rapid, easy, accurate and specific HPTLC method for quantitative estimation of kaempferol present in the Nyctanthes arbortristis Linn flowers has been developed and validated. HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The method used in this work resulted in good peak shape and enabled good resolution of kaempferol from other constituents of the plant material.

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