Qualitative and Quantitative Analysis of *Nyctanthes arbortristis* Linn leaf extracts by HPTLC

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Submission Date: 18-2-2014

Accepted Date: 24-3-2014

ABSTRACT

Nyctanthes arbortristis L. (Oleaceae) or "night jasmine" is cultivated for its fragrant flowers and is widely used in traditional systems of medicine as an anthelmintic, cholagogue, laxative and antimalarial. The plant has been studied pharmacognostically and certain standards are available. However there are no reports on HPTLC quality assessment of *Nyctanthes arbortristis*. Hence the present study involves the development of qualitative HPTLC fingerprint profile of n-hexane, ethyl acetate, methanol extracts of leaves of *N. arbortristis* followed by quantitation of marker compounds â-sitosterol, â-amyrin in n-hexane extract and caffeic acid in ethyl acetate extract. The developed methods were used for comparison of plant extracts with a few commercial formulations containing *N. arbortristis*. These HPTLC methods can be used easily for evaluation of quality of plants collected from different sources as well as for commercial formulations containing *N. arbortristis*.

Keywords: *Nyctanthes arbortristis,* commercial formulations*,* HPTLC qualitative and quantitative evaluation

INTRODUCTION

Nyctanthes arbortristis (Family: Oleaceae) commonly known as night jasmine or coral Jasmine (English) or Harsingh-har (Hindi) is a native of India and is widely distributed throughout India, Srilanka, Bangladesh and other parts of South East Asia.[1–4] It is cultivated all over the world for its fragrant flowers.[2] The bright orange corolla tubes of the flowers contain saffron - yellow colouring matter, this was formerly used for dyeing silk.[2,5,6]

Traditionally the plant was used by tribals (Santals) in snake bite and bites of wild animals. It was also used in cachexia, cancer, sores, ulcers, dysentery, and menorrhagia and enlargement of the spleen.[7] The bark has been used for treatment of bronchitis. The bark in combination with Arjun bark (*Terminalia arjuna*) was considered to be useful in internal injuries, healing of wounds including fractured bones.[8] Expressed juice of the leaves act as cholagogue, laxative and mild bitter tonic. Flowers have a bitter bad taste and have been used as a stomachic, carminative, astringent to the bowels. The powdered seeds were used to cure piles and skin diseases.[1,2,9–13] The decoction of leaves of this plant is widely used in Ayurvedic medicine for the treatment of sciatica, arthritis, as well as tonic, laxative and cholagogue.[1] Ethnobotanical surveys show that the plant has been used for prevention of malaria.[14]

The plant has been studied pharmacognostically and pharmacologically. [15,16] Phytochemical investigation of the plant has shown presence of different phytoconstituents in different parts of *N. arbortristis* plant (Table 1). Various extracts of the plant have shown different pharmacological activities (Table 2). Certain analytical standards for the plant extracts/constituents are available. Nyctanthic acid has been considered as one of the major constituents and it is estimated by TLC densitometric method.[15] Total glycosides have been estimated using TLC method.[17] Total phenolics and flavonoids were

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DOI: 10.5530/pj.2014.3.17

Table 1. Chemical constituents isolated from different parts of *Nyctanthes arbortristis*

Plant part	Chemical constituent	Reference
Bark	• A glycoside, • Two alkaloids one is soluble in water and other soluble in chloroform • Flavone glycoside	[2] [19]
Leaves	• Terpenoids Nyctanthic acid, Lupeol, Oleanolic acid, βamyrin, β-Sitosterol. • Phenolic compounds and flavanol glycosides Astragelin (kaempferol-3-glucoside), Nicotiflorin (kaempferol-3-rhamnoglucoside); Caffeic acid. • Iridoid glycosides 6β- hydroxyl loganin, Arborsides A, B, C and D, nyctanthoside Benzoic esters of loganin - 6,7-di- O- benzoylnyctanthoside, 6-O-trans-cinnamoyl- 6β- hydroxyl loganin, 7-O-trans-cinnamoyl-6β- hydroxyl loganin, Desrhamnosyl verbascoside • Carbohydrates D-mannitol, glucose, fructose. • N-alkanes Hentriacontane, tritriacontane	[20–22] [24;26–28] [29]
Flowers	• Oil This contains α pinene, p-cymene, 1-hexanol, methyl heptane, Phenyl acetaldehyde, 1-decanol and anisaldehyde. • Glycosides 6-O-trans cinnamoyl 7-O acetyl 6β- hydroxyl loganin, 6β- hydroxyl loganin , Arborside C and Nyctanthoside • Crocetin esters β -monogentiobioside ester of and β-digentiobioside ester of α-crocetin. • Benzofuranone derivatives 3, 3a, 7, 7a-tetra hydro-3a-hydroxy-6(2H)-benzofuranone • A carotenoid aglycone-crocetin	[30] [31] [32] [33] [34]
Seeds	• Iridoid glycosides Arbortristosides A, B, D, E. • Glycerides,Lignoceric acid, Stearic acid, Palmitic acid, myristic acids	[23; 25; 27] [35]

determined by spectrophotometrically.[17,18] However there were no reports on HPTLC profile of *N. arbortristis*. Hence the present study involves development of qualitative HPTLC fingerprint profile of n hexane, ethyl acetate, methanol extracts of leaves of *N. arbortristis* Linn along with HPTLC method for quantification of marker compounds β-sitosterol, β-amyrin in n-hexane extract and caffeic acid in ethyl acetate extract. These methods form the basis of standardization of commercial formulations containing *N. arbortristis*

MATERIALS AND METHODS

Plant Material

Leaves of *Nyctanthes arbortristis* were collected from Punjabi University campus (Patiala, India) during the months of February and March, 2007. The leaves were authenticated by Dr. H. B. Singh, Head, Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources (CSIR), New Delhi, 110067. A voucher specimen number: NISCAIR/RHMD/consult/2007-08/870/54 has been deposited in the same herbarium.

Chemicals

All chemicals used were of analytical grade and were purchased from S.D. Fine Chemicals Limited, Mumbai.

Refernce Standards

β-Sitosterol, β-amyrin and caffeic acid were gifted by National Institute of Pharmaceutical Education and Research (NIPER, Mohali).

Commercial Formulations

Three commercial formulations (Table 3) were procured from local market.

HPTLC equipment

A Camag TLC/HPTLC Integration (CATS V_4.04; S/N 0511A011); TLC Scanner 3 (V_1.14); Camag Linomat IV sample spotter; Camag glass twin trough chamber (20 cm × 10 cm)

Precoated Silica gel 60 TLC plate (0.2 mm thickness, 10 × 10 cm) (E. Merck); Temperature 25 ± 2°C, Relative humidity 40%

Table 2. Pharmacological activities reported from *Nyctanthes arbortristis*

Plant part	Extract/constituent	Activity observed	References
Leaves	Water soluble portion of the alcoholic extract	• Anti-inflammatory activity • Analgesic, antipyretic and ulcerogenic activities. • Prevent TNF- α accumulation in arthiritic and in induced lung injury in mice • Tranquilizing, depression of spontaneous motor activity, hypothermic and purgative activity	[37] [38] [39–40] [41]
	Ethanolic extract	• Trypanocidal activity • Bronchodilatory	[42] [43]
	Aqueous extract	Stimulation of Ach E activity in immuno suppresed mice	[44]
	• Methanolic extract • Phenolics of methanol extract	Anixolytic, nootropic and anti stress activity Anti oxidant activity	[45] [46]
	Acetone soluble fraction of ethyl acetate extracts.	Anti oxidant activity	[18; 47]
	Polyphenols from petroleum ether extract	Anti oxidant activity	[48]
	Alcoholic and aqueous extracts	Hepatoprotective activity	[49]
	β-sitosterol from petroleum ether extract	Analgesic and anti-inflammatory activity.	[50]
	Crude extract and RPHPLC fractions	Antiplasmodial activity	[51]
	Ethanol extract	Antimalarial	[52]
Stem Bark	Methanol extract of stem bark	Anti spermatogenic activity	[53]
Bark	Flavone glycoside	Anti-inflammatory	[19]
Flowers	Ethanolic extract and isolated carotenoid from extract.	Anti-inflammatory	[54]
	Chloroform and ethyl acetate extracts and isolated compound (3, 3a, 7, 7a - tetra hydro-3a-hydroxy-6(2H)-Benzofuranone	Antibacterial activity	[33; 36]
	Chloroform extract and its isolated compound NCS-2	Anti filarial activity	[56]
	4-hydroxyhexahydrobenzofuran-7-one	Cytotoxic activity	[55]
	Hot infusion	Moderate dose dependent sedative activity in male rats	[57]
Seeds	Iridoid glycosides (arbortristosides A, B, C and 6β hydroxyl loganin)	*In vivo* and *in vitro* anti leishmanial activity	[25; 58; 59]
	50% ethanolic extracts	Immunomodulatory activity	[60]
	Arbortristosides A and C	Significant mast cell stabilizing activity	[15]
	Ethanolic extracts and isolated compounds arbortristoside A, B	Anti viral activity	[61]
	Arbortristoside A	Anti-inflammatory and antinociceptive activity	[62]
Different parts	$CHCl_3$ and ethyl acetate extract of leaf, fruits, flowers, and seeds	Antibacterial against gram negative bacteria	[17]
	Water-soluble portion of the ethanol extracts of flowers, barks, seeds and leaves	CNS depressant activity	[63]
	Ethanolic extract of seeds and leaves	Diuretic	[64]

Preparation of leaf extracts

Fresh leaves were dried under shade at room temperature and powdered by electrical grinder. Powdered drug (120 g) was subjected to successive Soxhlet extraction with n-hexane, ethyl acetate and methanol. The extracts were collected and evaporated by Rotatory vacuum evaporator. The extractive values were calculated with reference to air dried drug.

Preparation of extracts of commercial formulations

a) From poly herbal formulation (Deep act OS)

Five tablets were powdered and 1 g each was used for extraction with n-hexane, ethyl acetate and methanol separately, by maceration. The resultant extracts were evaporated to dryness using a water bath. The extractive

Table 3. Constituents of commercial formulations containing *Nyctanthes arbortristis*

S.No	Formulations	Constituents	Manufacturer
1	Deep act OS tablets	Each tablet contains extracts of Shallaki 150mg Guggulu 120mg Parijatha 43mg Haridra 40mg Haritaki 25mg Shunthi 15mg	Lupin Herbal (Mumbai)
2	Mother tincture (1)	Alcoholic 59% v/v extract of *N.arbortristis*	Ralson Remedies (p) Ltd (Delhi)
3	Mother tincture (2)	Alcoholic 59% v/v extract of *N. arbortristis*	Medi synth.pvt. Ltd (Navi Mumbai)

values were calculated with reference to the weight of drug taken.

b) From mother tinctures

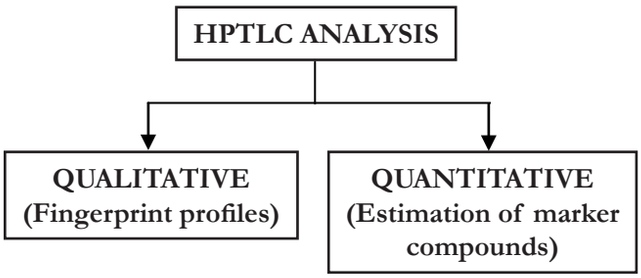
In the present study two mother tinctures of different companies were examined. Same extraction method was used for two mother tincture.

Mother tincture (50 ml) was evaporated to dryness by Rotavapour. The dried residue was subjected to successive fractionation by n-hexane, ethyl acetate and methanol by maceration. The extracts were evaporated to dryness by using a water bath. The extractive values were calculated with reference to the volume of the drug taken.

TLC profile of different extracts

The n-hexane, ethyl acetate and methanol extracts obtained from plant as well as commercial formulations were subjected to thin layer chromatographic (TLC) analysis. The solvent system of different extracts was given in the following (Table 4).

HPTLC Analysis



a) Qualitative analysis

Qualitative analysis was done by comparing Rf values, colour of the spot obtained, UV absorbance characters of the spots and fluorescent nature of the spots.

- n-hexane extracts

HPTLC profiles of n-hexane extracts of *N.arbotristis,* poly herbal formulation (Deep act OS) were developed and compared with standards β- sitosterol, β- amyrin.

- Ethyl acetate extracts

HPTLC profiles of ethyl acetate extracts of *N. arbotristis,* poly herbal formulation, mother tincture 1 and 2 were developed and compared with standard caffeic acid.

- Methanol extracts

HPTLC profile of methanol extracts of *N.arbotristis,* poly herbal formulation, mother tincture 1 and 2 were developed and compared with methanol extract of *N. arbotristis*.

Preparation of samples

From the dried residue of different extracts, the samples were prepared in the following way (Table 5).

Preparation of Standards

- β- Sitosterol - A stock solution of 0.1 mg/ml was prepared by dissolving 1 mg of β- sitosterol in 10 ml of hexane.
- β- Amyrin - A stock solution of 0.1 mg/ml was prepared by dissolving 1 mg of β- amyrin in 10 ml of hexane.
- Caffeic acid - A stock solution of 0.01 mg/ml was prepared by dissolving 1mg of caffeic acid in 100 ml of ethyl acetate.

Solvent system

The following solvent systems, derivatizing agents, and scanning wavelengths were used for different extracts (Table 6).

Method development

- n-hexane extracts

The n hexane extracts of *N. arbortristis* (2 µl), poly herbal formulation (1 µl) were applied in triplicate on precoated

Table 4. Different solvent systems used in TLC for different extracts

Samples	Solvent system	Derivatizing agent
n-hexane extracts (plant, poly herbal formulation)	Toluene: EtOAc (8:2)	Anisaldehyde sulphuric acid.
Ethyl acetate extracts (plant, poly herbal formulation, mother tincture 1 and 2)	EtOAc: formic acid: glacial acetic acid: H_2O (10:11:11:27)	NP-PEG reagent and observed under UV 366nm
Methanol extracts (plant, poly herbal formulation, Mother tincture 1 and 2)	$CHCl_3$: MeOH (8:2)	Vanillin in sulphuric acid

Table 5. Preparation of samples for qualitative HPTLC analysis

Extracts	Plant		PHF		MT_1		MT_2	
	stock solution	sample volume	stock solution	sample volume	stock solution	sample volume	stock solution	sample volume
n hexane	20 mg/ml	2 µl	20 mg/ml	1 µl	–	–	–	–
EtOAc	10 mg/ml	4 µl	10 mg/ml	4 µl	5 mg/ml	3 µl	5 mg/ml	3 µl
MeOH	20 mg/ml	4 µl	10 mg/ml	4 µl	10 mg/ml	4 µl	10 mg/ml	4 µl

PHF- Poly herbal formulation, MT1- Mother tincture 1, MT2- Mother tincture 2.

Table 6. Solvent systems used in HPTLC for different extracts

Extracts	Solvent system	Derivatizing agents	Scanning wave length
n-hexane	Toluene: EtOAc (8:2)	Anisaldehyde sulphuric acid	560 nm
EtOAc	Toluene: EtOAc: Acetic acid (5:4:1)	NP-PEG reagent	366 nm
MeOH	EtOAc: MeOH (8:2)	Anisaldehyde sulphuric acid	560 nm

EtOAc- Ethyl acetate, MeOH- Methanol, NP-PEG – Natural product- Poly ethylene glycol reagent.

silica gel TLC plate using linomat IV sample spotter. β-sitosterol (2 µl), β-amyrin (2 µl) were also applied on the same plate. The plates were developed in the solvent system mentioned in table 5 in glass twin trough chamber. The plates were developed up to 8cm. After development the plates were dried in air. Then the plates were derivatized with anisaldehyde sulphuric acid by dipping method. The plates were heated at 100ºC for 5 to 10 minutes. The plates were scanned at the wavelength of 560 nm.

- Ethyl acetate extracts

The ethyl acetate extracts of *N. arbortristis* (4 µl), poly herbal formulation (4 µl), mother tincture 1 and 2 (3 µl) were applied in triplicate on precoated silica gel TLC plate using linomat IV sample spotter. Caffeic acid (2 µl) was also applied on the same plate. The plates were developed in the solvent system mentioned in table 10 in glass twin trough chamber. The plates were developed up to 8cm. After development the plates were dried in air. Then the plates were derivatized with NP-PEG reagent by dipping method. The plates were scanned at the wavelength of 366 nm.

- Methanol extracts

The methanol extracts of *N. arbortristis* (4 µl), poly herbal formulation (4 µl), mother tincture 1 and 2 (4 µl) were applied in triplicate on precoated silica gel TLC plate using linomat IV sample spotter. The plates were developed in the solvent system mentioned in table 10 in glass twin trough chamber. The plates were developed up to 8 cm. After development the plates were dried in air. Then the plates were derivatized with anisaldehyde sulphuric acid reagent by dipping method. The plates were heated at 100ºC for 5 to 10 minutes. The plates were scanned at the wavelength of 560 nm.

b) Quantitative analysis

This involves quantitation of constituents by using calibration curves of standards. Amount or concentration was calculated by comparing peak area/peak height of sample with peak area/peak height of standard.

Development of calibration curves

- β-sitosterol (stock solution 0.1 mg/ml)

Aliquots of stock solution - 3 µl, 5 µl, 7 µl, 9 µl, 11 µl, 13 µl corresponding to 0.3 µg, 0.5 µg, 0.7 µg, 0.9 µg, 1.1 µg, 1.3 µg of β-sitosterol were applied on precoated silica gel 60 TLC plate. The plate was developed in solvent system (Toluene: ethyl acetate, 8:2) in glass twin trough chamber to a distance of 8 cm. After development the plate was dried in air. Then the plate was derivatized with

anisaldehyde sulphuric acid reagent by dipping method. The plate was heated at 100ºC for 5 to 10 minutes. The plate was scanned at the wavelength of 560 nm. The peak areas were recorded. The calibration curve of β-sitosterol was prepared by plotting peak areas versus concentration of β-sitosterol applied.

- β-amyrin (stock solution 0.1 mg/ml)

Aliquots of stock solution - 1 µl, 3 µl, 5 µl, 7 µl, 9 µl, corresponding to 0.1 µg, 0.3 µg, 0.5 µg, 0.7 µg, 0.9 µg of β-amyrin were applied on precoated silica gel 60 TLC plate. The plate was developed, derivatized, scanned as similar as β-sitosterol. The peak areas were recorded. The calibration curve of β-amyrin was prepared by plotting peak areas versus concentration of β-amyrin applied.

- Caffeic acid (stock solution 0.01 mg/ml)

Aliquots of stock solution (10 µl × 4 times), (20 µl × 2 times), corresponding to 0.1 µg, 0.2 µg of caffeic acid were applied on precoated silica gel 60 TLC plate. The plate was developed in solvent system (Toluene: EtOAc: acetic acid, 5:4:1) in glass twin trough chamber to a distance of 8 cm. After development the plate was dried in air. The plate was derivatized with NP-PEG reagent by dipping method. Then the plate was scanned by UV 366 nm. The peak areas were recorded. The calibration curve of caffeic acid was prepared by plotting peak areas versus concentration of caffeic acid applied.

Quantitation of β-sitosterol, β-amyrin in n-hexane extracts

β-sitosterol and β-amyrin was quantified in n-hexane extracts of *N. arbortristis* and poly herbal formulations. The plates were developed, derivatized and scanned as similar as qualitative analysis of n-hexane extracts. The peak areas were recorded. The concentration of β- sitosterol and β-amyrin were calculated by comparing peak areas with calibration curves of both.

Quantitation of caffeic acid in ethyl acetate extracts

Caffeic acid was quantified in ethyl acetate extracts of *N.arbortristis,* poly herbal formulation, mother tincture 1 and 2. The plates were developed, derivatized and scanned as similar as qualitative analysis of ethyl acetate extracts. The peak areas were recorded. The concentration of caffeic acid was calculated by comparing peak areas with calibration curve of caffeic acid.

RESULTS AND DISCUSSION

Procurement of leaves and preparation of extracts

Leaves of *N. arbortristis* were collected from Punjabi University campus and authenticated by NISCAIR, New Delhi, India as well as by critical morphological and microscopical examination and by comparison with standards available.[15] Commercial formulations, Deep Act OS (Lupin herbal), mother tinctures of *Nyctanthes arbortristis* from two different companies (Ralson Remedies, Medi Synth ltd) were purchased and used for our study. Three extracts (n hexane, ethyl acetate, and methanol) of each were prepared from the leaves of *N. arbortristis* and commercial formulations in order to separate the phytoconstituents on the basis of polarity. The n-hexane extracts are reported to contain β- sitosterol, β- amyrin.[20] The ethyl acetate extracts are reported to contain caffeic acid.[65]

Commercially available poly herbal formulation (Deep act OS) and mother tinctures containing *N. arbortristis* were processed. Sample preparation of both was designed in such a way so as to extract phytoconstituents, differentiated on the basis of polarity similar to the preparation of extracts from the plant material.

The percentage yields of different extracts of plant and commercial formulations were showed in (Table 7).

Thin layer chromatographic profiles

Results of thin layer chromatographic studies of different leaf extracts are reported in (Table 8).

Qualitative HPTLC analysis

Qualitative HPTLC examination revealed the presence of the marker compounds (β- sitosterol and β-amyrin) in n- hexane extract of plant and poly herbal formulation; caffeic acid in ethyl acetate extract of plant, poly herbal formulation, mother tincture 1 and 2 while in the methanol extracts, peak at Rf 0.34 was present in plant, mother tincture 1 and 2 and absent in poly herbal formulation. Peak at Rf 0.43 was present in plant, poly herbal formulation, mother tincture 1 and 2. This was done to confirm the presence of known marker compounds in plant material and commercial formulations.

HPTLC fingerprint profile of n-hexane extracts

The qualitative fingerprint profile of n-hexane extracts has been described using well knowns table compounds β-sitosterol, β-amyrin as marker compounds. The standards

Table 7. Percentage yields of extracts of plant and commercial formulations

S.No	Sample	n-hexane	Ethyl acetate	Methanol
1	*Nyctanthes arbortristis*	1.6% w/w	4.5% w/w	4.0% w/w
2	Poly herbal formulation (Deep act OS)	2.5% w/w	4.5% w/w	3.0% w/w
3	Mother tincture (1)	–	2.5% w/v	1.5% w/v
4	Mother tincture (2)	–	1.6% w/v	1.5% w/v

Table 8. TLC results of different extracts of leaves of *N. arbortristis* and its commercial formulations

Extract	Solvents with ratio	No. of spots	Rf Values
n-hexane extract of Plant PHF	Toluene:ethyl acetate, 8:2	3	0.52, 0.73, 0.78
		3	0.52, 0.69, 0.85
Ethyl acetate of Plant PHF MT_1 MT_2	Ethyl acetate:formic acid:glacial acetic acid:water,100:11:11:27	1	0.90
		1	0.90
		1	0.90
		1	0.90
Methanol extract of Plant PHF MT_1 MT_2	Chloroform:methanol,8:2	2	0.86, 0.87
		1	0.93
		2	0.78, 0.86
		2	0.56, 0.61

PHF- Poly herbal formulation, MT1- Mother tincture 1, MT2- Mother tincture 2.

β-sitosterol, β- amyrin were showed a purple coloured peak with the Rf Value 0.41, 0.54 respectively. The result of HPTLC analysis of n-hexane extract of the plant is detailed in plate 1, (Table 9).

The HPTLC profile of n-hexane extracts of *N. arbortristis* showed peak number 3, 5 at Rf value 0.40, 0.53 which correspond to β-sitosterol, β- amyrin respectively, thus showing presence of these well known compounds in the n- hexane extract of plant.

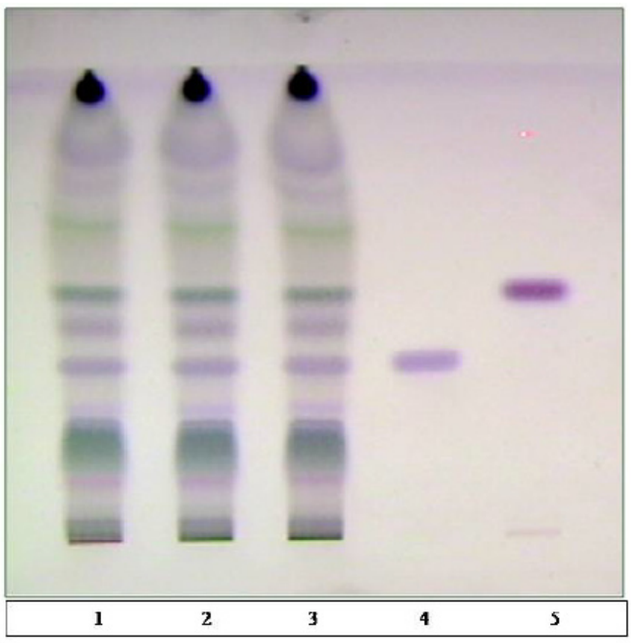
The following result was obtained with poly herbal formulation containing *N.arbortristis* (Plate 2, (Table 10).

The HPTLC profile of n-hexane extract of poly herbal formulation (Deep act OS) showed peak No 5, 7 at Rf value 0.40, 0.54 which correspond to β-sitosterol, β- amyrin respectively.

HPTLC fingerprint profile of ethyl acetate extracts

The qualitative fingerprint profile of ethyl acetate extracts has been described using caffeic acid as marker compound. The standard caffeic acid was showed light blue fluorescent peak with Rf value 0.53. The result of HPTLC analysis of ethyl acetate extract of plant is detailed in plate 3, (Table 11).

The HPTLC profile of ethyl acetate extracts of *N. arbortristis* showed peak No 2 at Rf value 0.52 and this corresponds to caffeic acid.The following results were obtained with commercial formulations containing *N. arbortristis* (Plates 4-5),(Table 12).

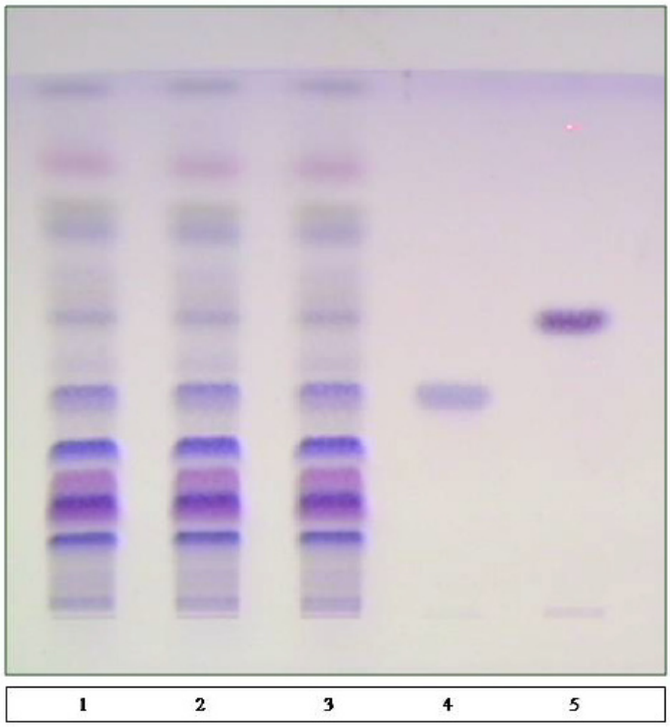


(1 - n hexane extract of plant; 2 - n -hexane extract of plant; 3 - n -hexane extract of plant; 4 - β sitosterol; 5 - β amyrin)

Plate 1. HPTLC profile of n hexane extract of plant and standards.

Table 9. HPTLC profile of n hexane extract of Plant and standards

Track No	Sample	Peaks	Rf value at max	Height of peak at max	AUC of peak
1,2,3	*Nycatanthes abortristis*	1	0.03	42.3	472.7
		2	0.20	217.9	16904.0
		3	0.40	97.1	3105.7
		4	0.43	87.8	2932.1
		5	0.53	141.8	3593.4
		6	0.64	34.9	1405.2
		7	0.72	17.7	439.8
		8	0.78	37.0	1477.7
4	β- sitosterol	1	0.40	162.9	6314.0
5	β- amyrin	1	0.54	313.5	10674.0

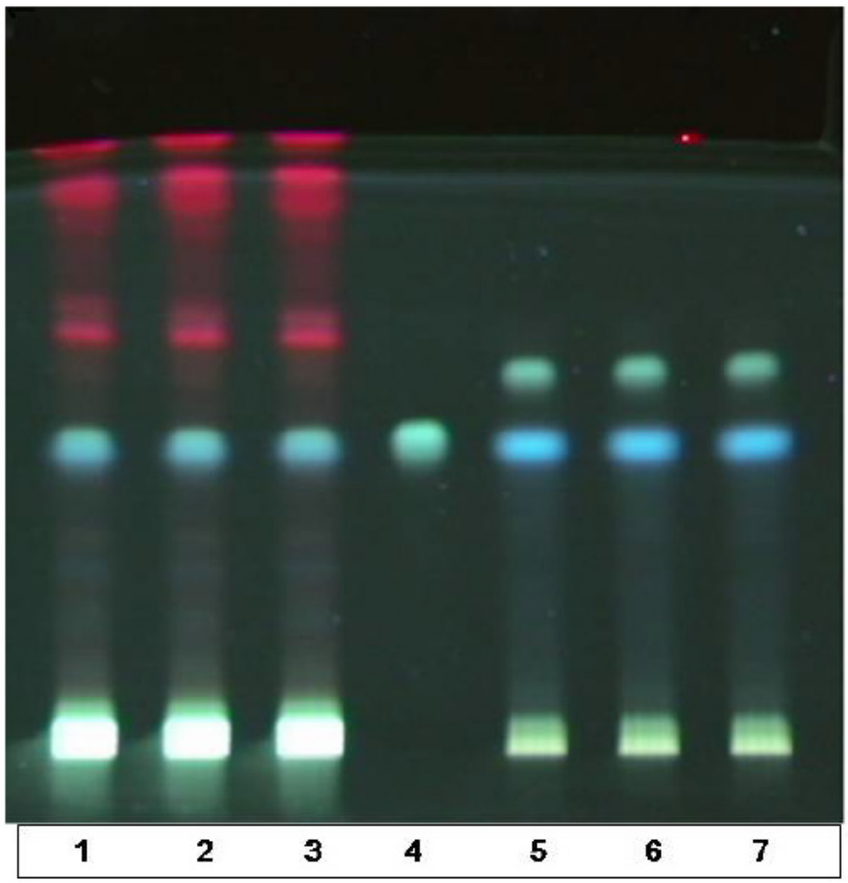


(1 - n hexane extract of PHF; 2 - n hexane extract of PHF; 3 - n hexane extract of PHF; 4 - β sitosterol; 5 - β -amyrin) (PHF - poly herbal formulation, Deep act OS)

Plate 2. HPTLC profile of n-hexane extract of PHF and standards.

Table 10. HPTLC profile of n hexane extracts of PHF and standards

Track No	Sample	Peaks	Rf value at max	Height of peak at max	AUC of peak
1,2,3	Poly herbal formulation (Deep act OS)	1	0.03	81.0	795.0
		2	0.14	265.5	7138.5
		3	0.20	240.3	13620.0
		4	0.31	289.3	7114.1
		5	0.41	172.6	5108.3
		6	0.46	24.3	466.9
		7	0.54	95.3	3290.3
		8	0.62	30.8	554.1
		9	0.71	100.6	4692.1
		10	0.83	80.7	2865.9
4	β-sitosterol	1	0.40	166.0	6557.6
5	β- amyrin	1	0.54	331.3	10939.5



(1 - EtOAc extract of plant; 2 - EtOAc extract of plant; 3 - EtOAc extract of plant; 4 - Caffeic acid; 5 - EtOAc extract of MT_1; 6 - EtOAc extract of MT_1; 7 - EtOAc extract of MT_1) (EtOAc – ethyl acetate; MT - mother tincture)

Plate 3. HPTLC of ethyl acetate extract of plant, Mother $Tincture_1$ and standard.

Table 11. HPTLC profile ethyl acetate of extract of plant and standard

Track No	Sample	Peaks	Rf value at max	Height of peak at max	AUC of peak
1,2,3	*Nyctanthes arbortristis*	1	0.09	11.9	84.5
		2	0.52*	44.6	1858.5
		3	0.68	71.6	3052.8
4	Caffeic acid	1	0.52	93.1	4468.2

* Blue fluorescent spot.

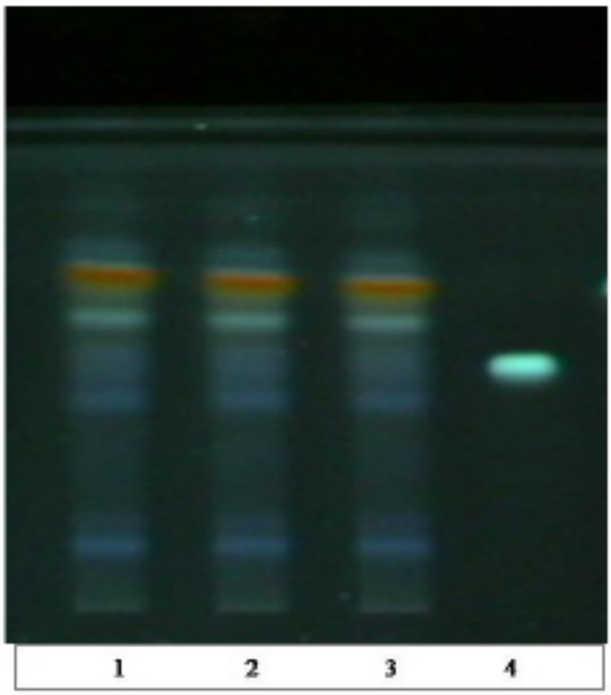
HPTLC fingerprint profile of methanol extracts

The HPTLC fingerprint profile of methanol extracts of commercial formulations have been described and compared with methanol extract of *N. arbortristis*. Here no external standard was used. The following result was obtained with methanol extract of plant and commercial formulations of plant (Plate 6), (Table 13).

From the results mentioned above it was observed that some related peaks at Rf value 0.34, 0.45 were present in the HPTLC profile of all four extracts. The colour of resolved bands in all four extracts was same under visible region. These may be due to the presence of same compounds, but the compounds cannot be specified in the absence of any standards.

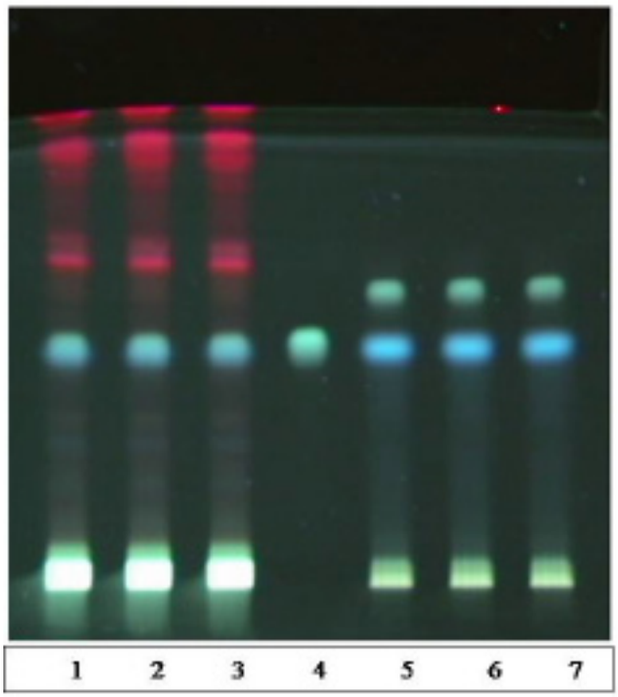
Quantitative HPTLC analysis

Although the presence of marker compounds (β- sitosterol, β-amyrin and caffeic acid) have been reported in the plant,[15,20,65] quantitative estimation had not been carried out to determine the amount of the well known marker compounds in the plant extracts. In the present study



(1 - EtOAc extract of PHF; 2 - EtOAc extract of PHF; 3 - EtOAc extract of PHF; 4 - Caffeic acid) (EtOAc – ethyl acetate; PHF – poly herbal formulation)

Plate 4. HPTLC profile ethyl acetate extract of Poly Herbal Formulation and standard.



(1 - EtOAc extract of plant; 2 - EtOAc extract of plant; 3 - EtOAc extract of plant; 4 - Caffeic acid; 5 - EtOAc extract of MT_2; 6 - EtOAc extract of MT_2, 7 - EtOAc extract of MT_2) (EtOAc – ethyl acetate; MT2 - Mother tincture2)

Plate 5. HPTLC profile of ethyl acetate extracts of plant, Mother tincture2 and standard.

Table 12. HPTLC profile of ethyl acetate extract of commercial formulations

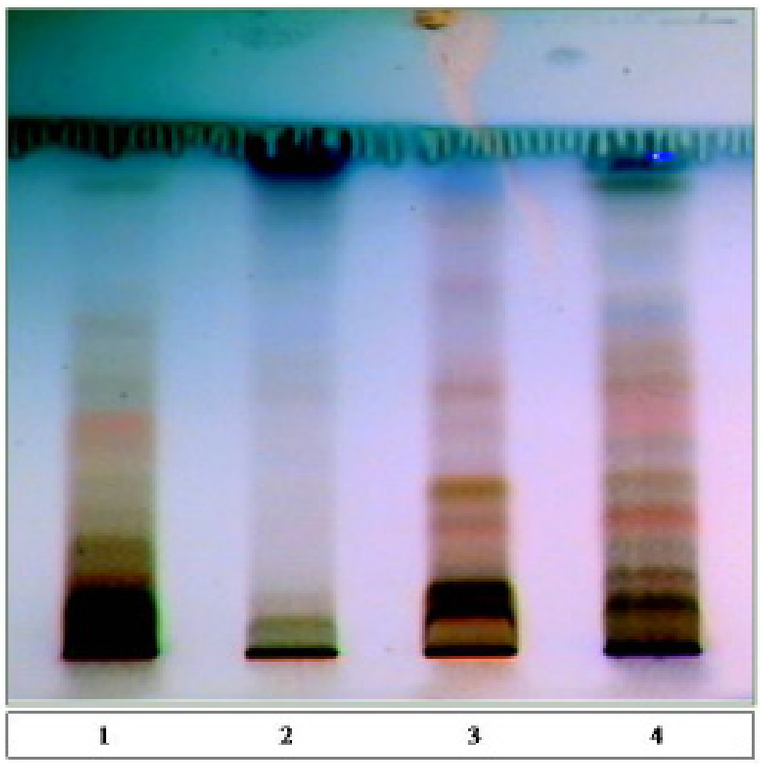
Plate	Track No	Sample	Peaks	Rf value at max	Height of peak at max	AUC of Peak
4	1,2,3	Poly herbal formulation. (Deep act OS)	1	0.12	46.3	1446.1
			2	0.40	65.7	4121.0
			3	0.52	67.8	3404.1
			4	0.55	118.0	3746.7
			5	0.63	97.5	3521.3
3	5,6,7	Mother tincture 1	1	0.51	25.7	1211.3
			2	0.62	60.0	2399.9
			3	0.68	21.2	634.6
5	5,6,7	Mother tincture 2	1	0.52	26.23	1646.7
			2	0.60	32.75	1840.6
			3	0.66	41.02	1585.4
5	4	Caffeic acid	1	0.52	85.0	4176.9

quantitation of β- sitosterol and β- amyrin was done in n hexane extract and caffeic acid in ethyl acetate extracts using standard plots of β-sitosterol, β-amyrin and caffeic acid.

Development of calibration curves

a) β-Sitosterol (0.1 mg/ml): Aliquots of stock solution - 3 µl, 5 µl, 7 µl, 9 µl, 11 µl, 13 µl corresponding to 0.3 µg, 0.5 µg, 0.7 µg, 0.9 µg, 1.1 µg, 1.3 µg of β-sitosterol were applied on precoated silica gel 60 TLC plate. The plate was developed in mobile phase (Toluene: ethyl acetate, 8:2) and scanned at 560 nm.

b) β-Amyrin (0.1 mg/ml): Aliquots of stock solution - 1 µl, 3 µl, 5 µl, 7 µl, 9 µl corresponding to 0.1 µg, 0.3 µg, 0.5 µg, 0.7 µg, 0.9 µg of β-amyrin were applied on



(1 - MeOH extract of plant; 2 - MeOH extract of PHF; 3 - MeOH extract of MT_1; 4 - MeOH extract of MT_2) (MeOH – methanol; PHF - poly herbal formulation; MT1 - Mother tincture 1; MT2 - Mother tincture 2)

Plate 6. HPTLC profile of methanol extract of plant and commercial formulations.

Table 13. HPTLC profile of methanol extract of plant leaves and commercial formulations

Track No	Sample	Peaks	Rf value at max	Height of Peak at max	AUC of Peak
1	Leaves	1	0.01	91.5	3400.9
		2	0.12	38.9	970.7
		3	0.34	14.2	533.3
		4	0.43	17.5	488.0
		5	0.86	25.1	466.9
2	PHF	1	0.02	13.5	199.7
		2	0.45	12.2	504.5
3	MT 1	1	0.02	255.6	4993.7
		2	0.03	211.2	4508.7
		3	0.17	47.1	1463.5
		4	0.24	87.0	2158.1
		5	*0.36*	15.0	408.3
		6	0.44	24.8	787.4
		7	0.66	16.2	629.5
		8	0.87	22.4	477.9
4	MT 2	1	0.01	121.8	3805.8
		2	0.06	88.8	960.5
		3	0.12	22.0	396.2
		4	0.19	55.3	1347.4
		5	0.26	42.4	911.7
		6	0.33	26.5	673.8
		7	0.45	51.3	2407.2
		8	0.51	39.5	838.9
		9	0.60	60.6	2617.5
		10	0.73	14.3	501.8
		11	0.86	79.3	1621.8

PHF- Poly herbal formulation, MT1- Mother tincture 1, MT2- Mother tincture 2.

precoated silica gel 60 TLC plate. The plate was developed, derivatized, scanned as similar as β-sitosterol.

c) Caffeic acid (0.01 mg/ml): Aliquots of stock solution (10 µl × 4 times), (20 µl × 2 times), corresponding to 0.1 µg, 0.2 µg of caffeic acid were applied on precoated silica gel 60 TLC plate. The plate was developed in mobile phase (Toluene: EtOAc: acetic acid, 5:4:1) and scanned by UV 366 nm.

The calibration curves for different standards were studied by linear regression method. Quantitative analysis of markers in different extracts was carried out. The amount of β- sitosterol and β-amyrin was determined using calibration of curve of standards and area under the curve (AUC) of the n- hexane extract of plant and in PHF and the amount of Caffeic acid in ethyl acetate extracts of plant and in commercial formulations was determined using AUC of plant, MT_1, PHF and MT_2 (Tables 14–16).

The study has revealed the quantities of marker compounds in different extracts of plant and commercial formulations (Table 17). The commercial formulations studied in this study contained the marker compounds in quantities greater than that of the plant extracts.

CONCLUSIONS

HPTLC method for qualitative evaluation and quantitative estimation of marker compounds in extracts was found to be simple, precise, specific, and sensitive and can be used for routine quality control of plant material and commercial formulation of the plant.

ACKNOWLEDGEMENTS

Authors thankfully acknowledge the facilities provided by the Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala.

Table 14. Analysis of n-hexane extracts

Sample	Qualitative Analysis (presence/absence)		Quantitative Analysis Mean(%w/w of marker) ± S.D.	
	β- sitosterol	β- amyrin	β- sitosterol	β- amyrin
Leaves	+	+	0.021 ± 0.0017	0.016 ± 0.0007
PHF	+	+	0.133 ± 0.0043	0.046 ± 0.0015
MT1	–	–	–	–
MT2	–	–	–	–

PHF- Poly herbal formulation, MT1- Mother Tincture 1, MT2- Mother tincture 2.

Table 15. Analysis of ethyl acetate extracts

Sample	Qualitative Analysis (presence of caffeic acid)	Quantitative Analysis Mean (% w/w of caffeic acid) ± S.D.
Leaves	+	0.013 ± 0.0015
PHF	+	0.020 ± 0.0007
MT1	+	0.013 ± 0.001
MT2	+	0.010 ± 0.0007

PHF- Poly herbal formulation, MT1- Mother tincture 1, MT2- Mother tincture 2.

Table 16. Qualitative analysis of methanol extracts

Sample	Number of Peaks	Peak at Rf 0.3	Peak at Rf 0.43
Leaves	5	Present	Present
PHF	2	Absent	Present
MT1	8	Present	Present
MT2	11	Present	Present

PHF- Poly herbal formulation, MT1- Mother tincture 1, MT2- Mother tincture 2.

Table 17. Quantitative analysis of various extracts

Sample	Percentage of marker compounds in prepared extracts		
	n-hexane extract		Ethyl acetate extract
	β -Sitosterol	β -Amyrin	Caffeic acid
N. arbortristis	0.021 ± 0.0017	0.016 ± 0.0007	0.013 ± 0.0015
PHF	0.133 ± 0.0043	0.046 ± 0.0015	0.020 ± 0.0007
Mother tincture 1	–	–	0.013 ± 0.001
Mother tincture 2	–	–	0.010 ± 0.0007

PHF poly herbal formulation.

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