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Original Article

PHYTOCHEMICAL SCREENING AND HPTLC FINGERPRINTING ANALYSIS OF ETHANOLIC EXTRACT OF *ERYTHRINA VARIEGATA* L. FLOWERS

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ABSTRACT

Objective: Medicinal plants possess phytochemicals that accumulate in various parts of plant like leaves, flowers, vegetables and roots that exert defense mechanism and protect us from various diseases. The present study was carried out to investigate the secondary metabolites and develop HPTLC fingerprinting of *Erythrina variegata* L.

Methods: The Phytochemical screening was analyzed for various extracts by using standard protocols and HPTLC analysis was carried out for the identification of alkaloids, flavonoids, glycosides, saponin and steroids in the ethanolic extract of *Erythrina variegata* L. flower.

Results: The phytochemicals screening confirms the presence of phyto-constituents in various plant extracts of *Erythrina variegata* L. The study revealed the presence of alkaloids, flavonoids, glycosides, saponin and steroids in the ethanolic extract of *Erythrina variegata* L. flower.

Conclusion: Based on the study it is concluded that *Erythrina variegata* L. flower possess phytochemicals like alkaloids, flavonoids, glycosides, saponin and steroids.

Keywords: *Erythrina variegata* L., HPTLC, Medicinal plants, Phytoconstituents, Secondary metabolites

INTRODUCTION

Plants are naturally gifted tool and the extractions, characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic values [1]. Herbal extracts can act in a synergistic manner within the human body and provide unique therapeutic properties with minimal or no undesirable side effects [2]. Phytochemistry is the subject that deals with chemicals derived from plants. There are a large number of secondary metabolic compounds found in plants [3]. Different phytoconstituents of herbal products are safer than synthetic medicine and beneficial in the treatment of diseases caused by free radicals and it also protects the body from tissue injury [4]. Phytochemicals comprise primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds are terpenoid, alkaloids and phenolic compounds [5]. HPTLC and HPLC, both emerged efficient tools for phytochemical evaluation, and enable the analysis of several samples simultaneously. It reduces both time and cost of analysis [6]. The same plate can be visualized in several ways and individual spots can be quantitatively determined by densitometry in a specific track, called a fingerprint [7-9].

The genus Erythrina comprises about 110 species of trees and shrubs. The name "coral tree" is used as a collective term for these plants. Coral tree is indigenous to the Old World tropics, possibly originated from India to Malaysia, but is native of ancient Westward to Zanzibar and Eastward to Eastern Polynesia [10]. *Erythrina variegata* L. (Family-fabaceae) is a medium sized quick growing tree found in deciduous forests throughout India [11]. Various parts of this plant are used in traditional folk medicine across Asia, for treating ailments from liver trouble to leprosy. Its extracts exhibit sedative, antidiuretic, antihyperlipidemic and antiulcer activities [12]. The objective of the present study was to find phyto-constituents in different parts of *Erythrina variegata* L.

MATERIALS AND METHODS

Plant collection and authentication

The fresh plant samples were collected in the month of August, from in and around Kodaikannal, Dindigul district, Tamil Nadu, India. They were botanically authenticated by Dr. G. V. S Moorthy, Botanical Survey of India, TNAU campus, Coimbatore. The specimen was deposited in the Herbarium for future reference (voucher number: BSI/SRC/5/23/2013-14/Tech/1500). The plant samples were thoroughly washed under running tap water to remove adhering dust particles and blotted dry under shade for about two weeks, ground into milled powder and stored in an airtight container used for further investigations.

Preparation of extracts

The powdered plant samples of leaves, flowers and barks (100 g) were used for successive solvent extraction (500 ml) with increasing order of polarity like petroleum ether, chloroform, ethyl acetate, ethanol and water. The extraction was carried out for 48 h. The extract was concentrated by a Rotary flask evaporator. Each time before extracting with the next solvent the residue was dried thoroughly to remove the solvent used. The extracted samples were collected and used for phytochemical screening.

Preparation of ethanolic extract of flowers

100 g of powdered flowers was weighed and extracted with 500 ml of ethanol. Then it was kept in an orbital shaker at 190-220 rpm for 48 h. The supernatant was collected, filtered through Whatman No.1filter paper and then concentrated by evaporating to dryness which give a solid amorphous residue. The obtained dried extract was then accurately weighed, stored in small vials at-20 °C and used for the following studies.

Phytochemical screening of phytoconstituents

Phytochemical screening was carried out to evaluate the qualitative chemical composition of various crude extracts to identify the major primary and secondary metabolites groups such as steroids, reducing sugars, alkaloids, phenolic compounds, saponins, tannins, flavonoids, amino acids, terpenoids and cardioglycosides by using precipitation and coloration methods. This analysis revealed the presence or absence of these compounds in the crude extracts tested 13-14] and the yields of plant material in different solvents were also recorded.

HPTLC finger printing analysis of ethanolic extract of *Erythrina variegata* L. flowers

Test solution preparation

The plant samples 10 mg was weighed in an electronic balance (Afcoset) dissolved with 250 µl of respective solvent and centrifuged at 3000rpm for 5 min. This solution was used as a test solution for HPTLC analysis [15].

Sample application

2 µl of test solution and 2 µl of standard solution were loaded as 5 mm band length in the 10 x 10 Silica gel $60F_{254}$ TLC plate using a Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (over saturated with solvent vapor) with respective mobile phase (alkaloid, flavonoids, glycosides, saponins and steroid) and the plate was developed in the same respective mobile phase up to 90 mm (indicated below).

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and it captured the images in visible light, UV 254 nm and UV366 nm.

Derivatization

The developed plate was sprayed with respective spray reagent (alkaloid, flavonoids, glycosides, saponins and steroid) (indicated below) and dried at 100 °C in a Hot air oven. The plate was photo-documented in visible light and UV 366 nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning

After derivatization, the plate was fixed on scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500 nm. The Peak table, Peak display, and Peak densitogram were noted. The software used was won CATS 1.3.4 version.

Analysis of alkaloid, flavonoids, glycosides, saponins and steroids

Mobile phase

Alkaloids: Ethyl acetate-methanol-water (10: 1.35: 1).

Flavonoids: Ethyl acetate-Butanone-Formic acid-Water (5:3:1:1).

Glycosides: Ethyl acetate-Ethanol-Water (8:2:1.2).

Saponins: Mobile phase: Chloroform–Glacial acetic acid–Methanol–Water (6.4:3.2:1.2:0.8).

Steroids: Mobile phase: Toluene-Acetone (9:1).

Spray reagent

Alkaloids: Dragendorff's reagent followed by 10% Ethanolic sulphuric acid reagent.

Flavonoids: 1% Ethanolic Aluminium chloride reagent

Glycosides: Liberman-Burchard reagent.

Saponins: Anisaldehyde sulphuric acid reagent.

Steroids: Anisaldehyde sulphuric acid reagent.

RESULTS AND DISCUSSION

Phytochemicals are products of plant metabolism, mainly used by the plants for their defense. Hence, attempts have been made to use them for therapeutic purposes [16]. The phytochemical screening of the medicinal plants are also important and have a commercial interest in both research institutes and pharmaceutical companies in the manufacturing of the novel drugs used for treatment of various diseases [5].

The preliminary phytochemical screening of *Erythrina variegata* L. leaves, flowers and barks showed the presence of carbohydrates, proteins, amino acids, alkaloids, flavonoids, glycosides, saponins, steroids, tannins and terpenoids. The results of phytochemical analysis are tabulated in table 1, 2 and 3. In the phytochemical investigation, ethanolic extract of *Erythrina variegata* L. flowers confirmed more amounts of phytoconstituents while comparing to leaf and bark extracts. Therefore ethanolic extract of *Erythrina variegata* L. flower was used for further studies.

Percentage of yield

The percentage of the yield of various parts and extracts were tabulated in table 4.

HPTLC analysis

HPTLC is a powerful analytical technique. This method is visual, rapid and economical as it utilizes smaller amounts of solvents with minimum sample clean up. Above all, in a short duration a large number of samples are analyzed simultaneously [17]. HPTLC profile of ethanolic extract of *Erythrina variegata* L. flowers was recorded in tables 5, 6, 7, 8 & 9 and fig. 1-10 for alkaloids, flavonoids, glycosides, saponins and steroids respectively. The extracts were run along with the standards such as colchicine, rutin, swertiamarin, saponin 1, 2, & 3 and stigmasterol respectively.

Table 1: Phytochemical screening of *Erythrina variegata* L. leaves

Solvent extraction	AL	FL	TP	AP	CH	CG	SA	OF	TN	ST
Petroleum ether	+	-	-	+	+	-	-	-	-	-
Chloroform	-	-	-	+	+	-	-	-	-	-
Ethyl acetate	+	+	-	+	+	-	-	-	-	-
Ethanol	+	+	+	+	+	+	+	-	-	+
Water	+	+	-	+	+	+	-	-	-	+

Table 2: Phytochemical screening of *Erythrina variegata* L. flowers

Solvent extraction	AL	FL	TP	AP	CH	CG	SA	OF	TN	ST
Petroleum ether	+	-	-	+	+	+	-	-	-	-
Chloroform	-	-	-	+	+	+	-	-	-	-
Ethyl acetate	-	+	-	+	+	+	-	-	-	-
Ethanol	+	+	+	+	+	+	+	+	-	+
Water	+	+	-	+	+	+	-	-	+	+

Table 3: Phytochemical screening of *Erythrina variegata* L. bark

Solvent extraction	AL	FL	TP	AP	CH	CG	SA	OF	TN	ST
Petroleum ether	+	+	-	+	-	+	+	+	-	-
Chloroform	-	+	-	+	+	-	-	+	-	-
Ethyl acetate	-	+	-	+	+	+	+	+	-	-
Ethanol	+	+	-	+	+	+	-	-	-	+
Water	+	+	+	+	+	+	-	-	-	+

AL-	Alkaloids	CG-	Cardioglycosides
SA-	Saponins	OF-	Oils and Fats
TP-	Tannin and phenolic compounds	TN-	Terpenoids
FL-	Flavonoids	AP-	Amino acids and Proteins
ST-	Steroids	CH-	Carbohydrates
"+" Present		"-" Absent	

Table 4: Percentage yields of *Erythrina variegata* L.

S. No.	Solvents	% Yields of *Erythrina variegata* L.(g)		
		Leaves	Flowers	Bark
1	Petroleum ether	4.202	0.664	0.413
2	Chloroform	2.296	1.22	1.267
3	Ethyl acetate	1.176	1.068	0.931
4	Ethanol	1.062	1.186	0.783
5	Water	4.568	5.404	4.10

Table 5: Peak table of alkaloids and unknown compounds in ethanolic extract of *Erythrina variegata* L. flowers

Track	Peak	R_f	Height	Area	Assigned substance
STD	1	0.53	101.0	2217.3	Colchicine
Sample EV. F	1	0.04	14.9	120.5	Unknown
Sample EV. F	2	0.07	69.1	756.3	Unknown
Sample EV. F	3	0.09	99.7	2983.5	Alkaloid/Nitrogen-containing compound 1
Sample EV. F	4	0.14	85.3	2051.9	Alkaloid/Nitrogen-containing compound 2
Sample EV. F	5	0.18	158.7	5029.1	Alkaloid/Nitrogen-containing compound 3
Sample EV. F	6	0.22	108.8	2624.2	Unknown
Sample EV. F	7	0.28	221.6	6063.1	Unknown
Sample EV. F	8	0.33	241.8	5752.7	Alkaloid/Nitrogen-containing compound 4
Sample EV. F	9	0.36	195.9	4053.2	Alkaloid/Nitrogen-containing compound 5
Sample EV. F	10	0.40	58.6	689.5	Unknown
Sample EV. F	11	0.41	482.2	610.0	Unknown
Sample EV. F	12	0.47	94.1	2396.2	Alkaloid/Nitrogen-containing compound 6
Sample EV. F	13	0.55	39.5	1120.5	Alkaloid/Nitrogen containing compound 7
Sample EV. F	14	0.59	33.1	250.0	Unknown
Sample EV. F	15	0.63	106.9	3017.1	Unknown
Sample EV. F	16	0.75	132.7	3826.8	Unknown
Sample EV. F	17	0.82	21.3	207.3	Unknown
Sample EV. F	18	0.87	23.0	217.5	Unknown

EV. F-*Erythrina variegata* L. flowers & STD: Standard

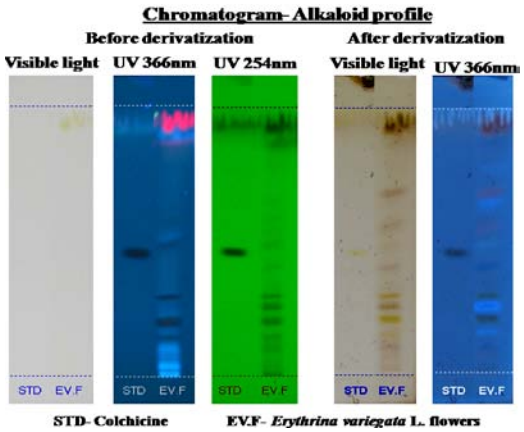
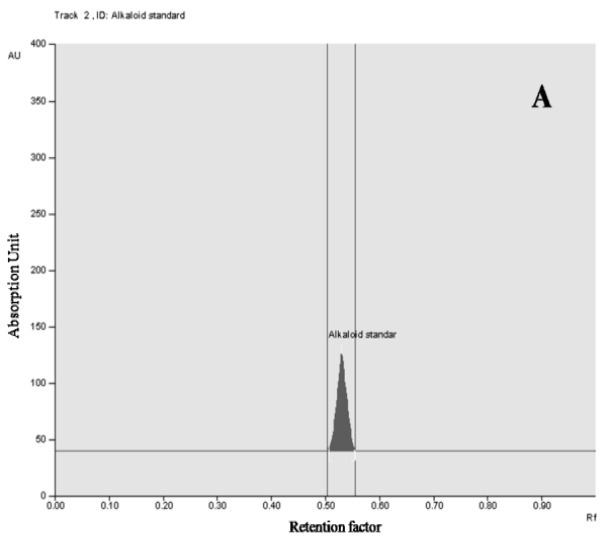
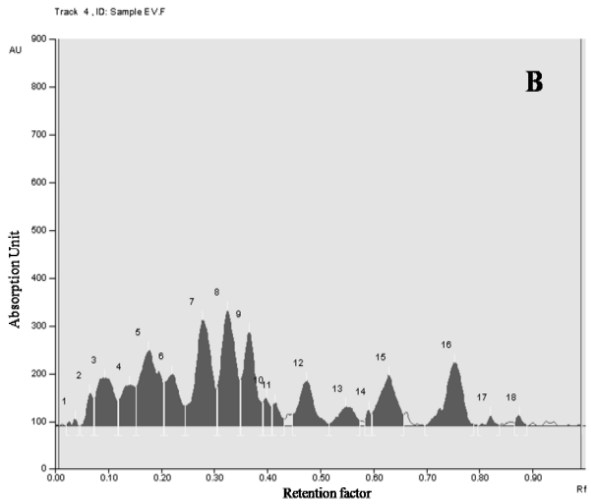


Fig. 1: Chromatograms of ethanolic extract of *Erythrina variegata* L. flowers in HPTLC analysis-Before derivatization under visible light, UV 366 nm and UV 254 nm. After derivatization under visible light and UV 366 nm





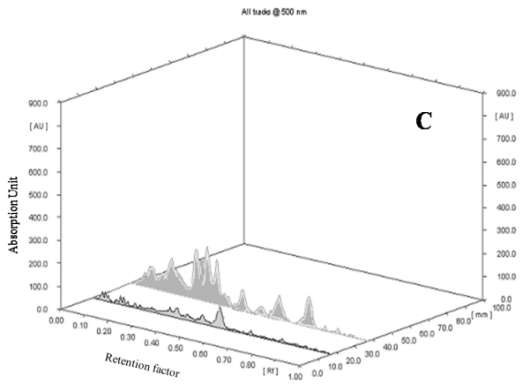


Fig. 2: Densitogram and 3D display for alkaloid profile of ethanolic extract *E. variegata* L. flowers and standard. A& B-Densitogram of alkaloid standard and *E. variegata* L. flowers, C-3D display of alkaloid standard and *E. variegata* L. flowers

Table 6: Peak table of flavonoid compounds in ethanolic extract of *Erythrina variegata* L. flowers

Track	Peak	R_f	Height	Area	Assigned substance
STD	1	0.51	464.3	20365.2	Rutin
Sample EV. F	1	0.08	104.3	2338.9	Unknown
Sample EV. F	2	0.12	91.1	2295.9	Unknown
Sample EV. F	3	0.18	200.4	8814.5	Flavonoid 1
Sample EV. F	4	0.27	423.2	18292.4	Flavonoid 2
Sample EV. F	5	0.39	269.6	11469.8	Flavonoid 3
Sample EV. F	6	0.53	20.6	462.3	Unknown
Sample EV. F	7	0.80	94.4	3366.3	Flavonoid 4
Sample EV. F	8	0.91	64.1	2026.4	Flavonoid 5

EV. F-*Erythrina variegata* L. flowers & STD: Standard

From the sample of *Erythrina variegata* L. flowers, 7 alkaloid compounds with Rf values of 0.09, 0.14, 0.18, 0.33, 0.36, 0.47, 0.55 were detected along with 11 unknown compounds (table 1). In chromatogram (fig. 1), Orange, yellow and brownish-yellow coloured zone at visible mode was observed in the tracks which after derivatization confirmed the presence of alkaloid/nitrogen containing the compound in the samples. In fig. 2 densitogram and 3D display for alkaloid profile of *E. variegata* L. flowers and standard are displayed. Alkaloids comprise one of the major groups of plant constituents. Several of the alkaloids were in clinical use, including reserpine (the first tranquilizer) and the dimeric indole alkaloids vinblastine and vincristine (anticancer agents) [18].

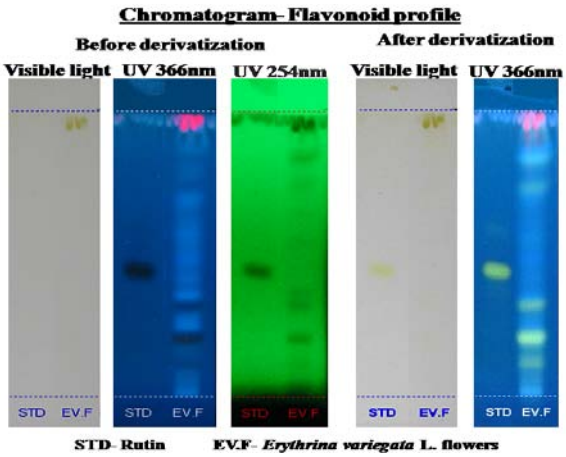
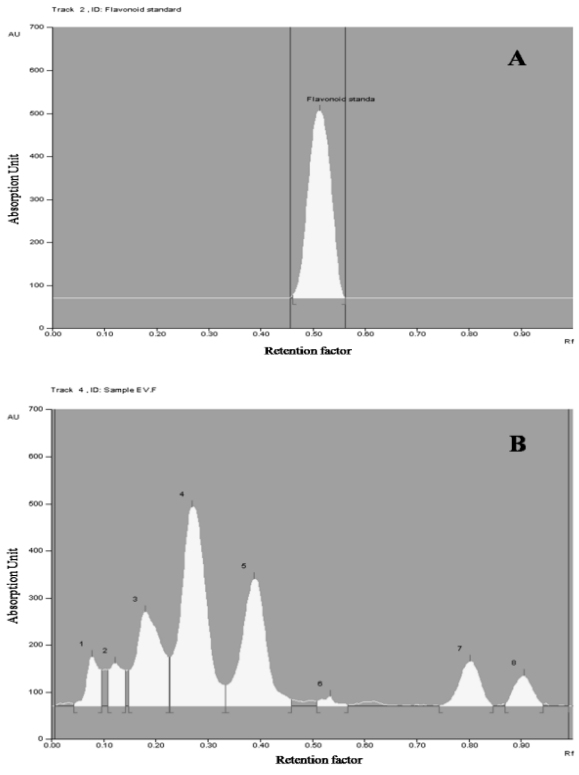


Fig. 3: Chromatograms of ethanolic extract of *Erythrina variegata* L. flowers in HPTLC analysis-Before derivatization under visible light, UV 366 nm and UV 254 nm. After derivatization under visible light and UV 366 nm

The alkaloid is primarily found in higher plants, but also in lower organisms and even in some animals and exhibit significant pharmacological activity. They contain some of the most complicated molecular structures and represent one of the largest and most diverse families of natural compounds [19].



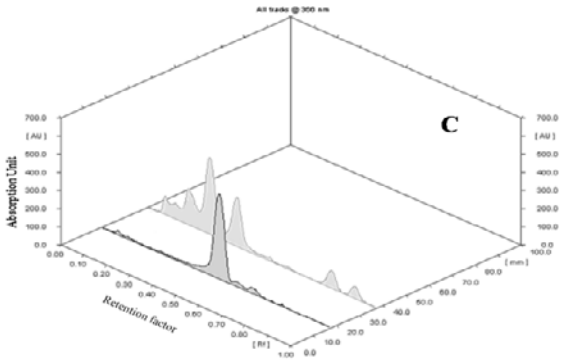


Fig. 4: Densitogram and 3D display for flavonoid profile of ethanolic extract of *E. variegata* L. flowers and standard. A and B-Densitogram of flavonoids standard and *E. variegata* L. flowers, C-3D display of flavonoids standard and *E. variegata* L. flowers

HPTLC of the ethanolic extract of *Erythrina variegata* L. flowers (table 6) show eight peak areas, thereby showing eight R_f values, thus it illustrates the presence of eight different constituents in *Erythrina variegata* L. flowers. Among them the peaks 3, 4, 5, 7 & 8 showed the presence of flavonoids. Fig. 4 exhibited the densitogram and 3D display for a flavonoid profile of *E. variegata* L. flowers. Yellow and yellowish blue coloured fluorescent zone at the UV 366 nm mode after derivatization confirms the presence of flavanoids in the sample (fig. 3). Flavonoids are the most important natural phenolic and they possess a broad spectrum of chemical and biological activities including free radical scavenging properties [20]. The flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti inflammatory, anti carcinogenic etc [21].

Brown and brownish yellow coloured zones at the visible light mode are present in the specified standard and sample tracks are indicated in the chromatogram after derivatization (fig. 5) thus confirmed the presence of glycoside in the known standard and in the samples. Table 7 documented the presence of 6 glycoside compounds. The densitogram and 3D display for glycoside profile of *E. variegata* L. flowers and standard with the peak values were shown in fig. 6. Glycosides comprise a very wide range of compounds that are common and ubiquitous occurrence in almost all plants. Many plants stored the medicinally important chemicals in the form of active glycosides that act as cardiac drugs, laxatives, counterirritants, analgesics, renal disinfectants, antirheumatics, anti-inflammatory, antituberculosis, expectorant [15].

Table 7: Peak table of glycosides and unknown compounds in ethanolic extract of *Erythrina variegata* L. flowers

Track	Peak	R_f	Height	Area	Assigned substance
STD	1	0.67	252.6	8363.1	Swartiamarin
Sample EV. F	1	0.02	17.0	183.2	Unknown
Sample EV. F	2	0.07	98.7	2534.8	Unknown
Sample EV. F	3	0.13	98.5	2780.4	Unknown
Sample EV. F	4	0.16	70.4	1361.3	Unknown
Sample EV. F	5	0.21	105.0	2871.2	Unknown
Sample EV. F	6	0.25	235.0	6863.4	Glycoside 1
Sample EV. F	7	0.31	111.5	4096.2	Unknown
Sample EV. F	8	0.46	160.5	10694.4	Glycoside 2
Sample EV. F	9	0.58	60.9	2409.7	Glycoside 3
Sample EV. F	10	0.70	17.6	552.9	Unknown
Sample EV. F	11	0.76	93.0	2085.3	Glycoside 4
Sample EV. F	12	0.79	43.9	832.5	Unknown
Sample EV. F	13	0.86	44.0	840.4	Glycoside 5
Sample EV. F	14	0.92	84.5	1901.1	Glycoside 6

EV. F-*Erythrina variegata* L. flowers & STD: Standard

Table 8: Peak table of saponins and unknown compounds in ethanolic extract of *Erythrina variegata* L. flowers

Track	Peak	R_f	Height	Area	Assigned substance
STD 1	1	0.25	108.5	3139.9	Saponin 1
STD 2	2	0.27	108.7	3299.0	Saponin 2
STD 3	3	0.44	43.3	2651.9	Saponin 3
Sample EV. F	1	0.01	39.4	332.4	Unknown
Sample EV. F	2	0.03	16.5	151.6	Unknown
Sample EV. F	3	0.07	34.3	549.4	Unknown
Sample EV. F	4	0.11	28.4	181.7	Unknown
Sample EV. F	5	0.12	14.0	105.7	Unknown
Sample EV. F	6	0.18	19.3	131.3	Unknown
Sample EV. F	7	0.23	21.4	222.8	Saponin 1
Sample EV. F	8	0.25	17.1	449.0	Unknown
Sample EV. F	9	0.32	19.1	284.5	Unknown
Sample EV. F	10	0.43	36.4	677.2	Saponin 2
Sample EV. F	11	0.47	56.0	2148.3	Saponin 3
Sample EV. F	12	0.54	67.9	1295.5	Unknown
Sample EV. F	13	0.55	65.6	2201.6	Unknown
Sample EV. F	14	0.76	38.7	1200.4	Saponin 4
Sample EV. F	15	0.88	73.4	1821.5	Unknown
Sample EV. F	16	0.97	104.9	2442.7	Unknown

EV. F-*Erythrina variegata* L. flowers & STD: Standard

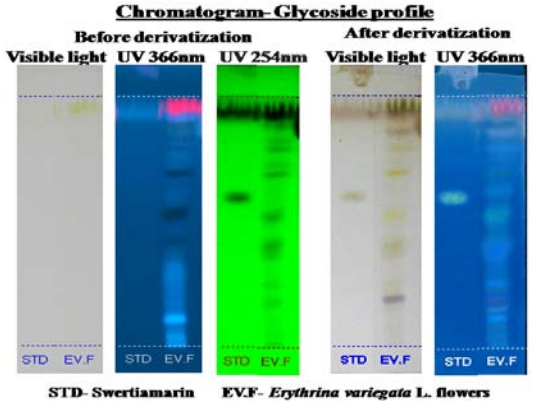


Fig. 5: Chromatograms of extract in HPTLC analysis-Before derivatization under visible light, UV 366 nm and UV 254 nm. After derivatization under visible light and UV 366 nm

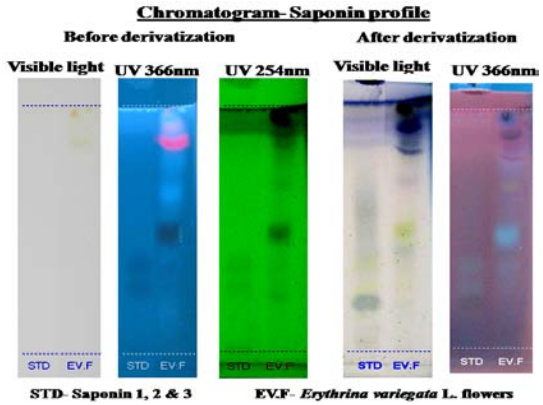


Fig. 7: Chromatograms of ethanolic extract of *Erythrina variegata* L. flowers in HPTLC analysis-Before derivatization under visible light, UV 366 nm and UV 254 nm. After derivatization under visible light and UV 366 nm

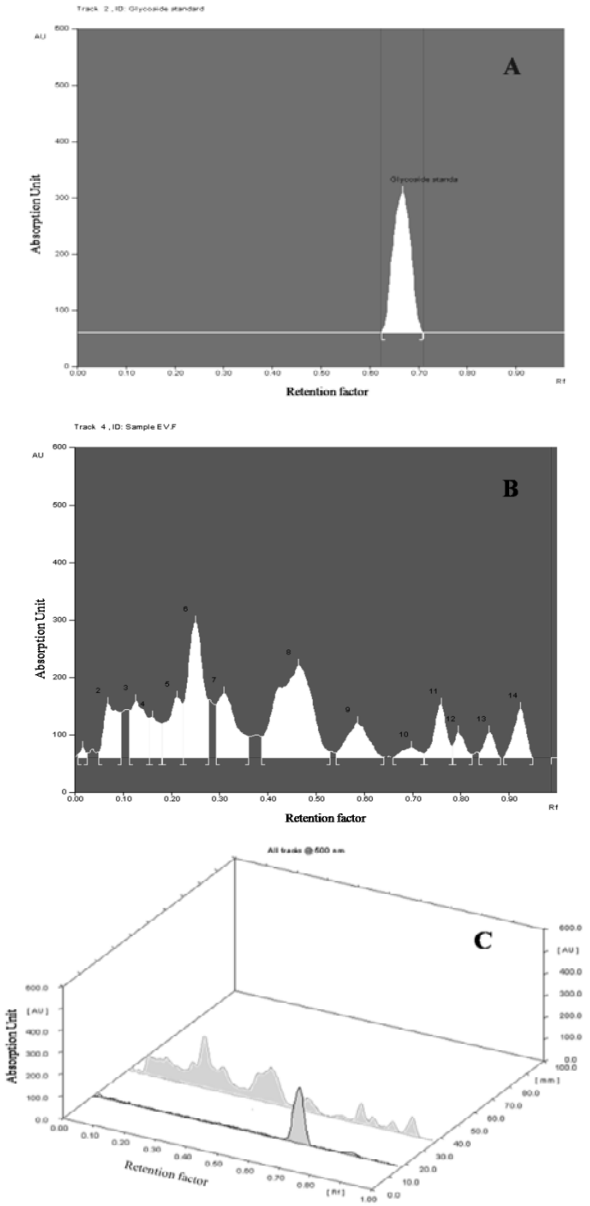


Fig. 6: Densitogram and 3D display of glycoside profile of ethanolic extract of *E. variegata* L. and standard. A and B-Densitogram of glycosides standard and *E. variegata* L. flowers, C-3D display of glycosides standard and *E. variegata* L. flowers

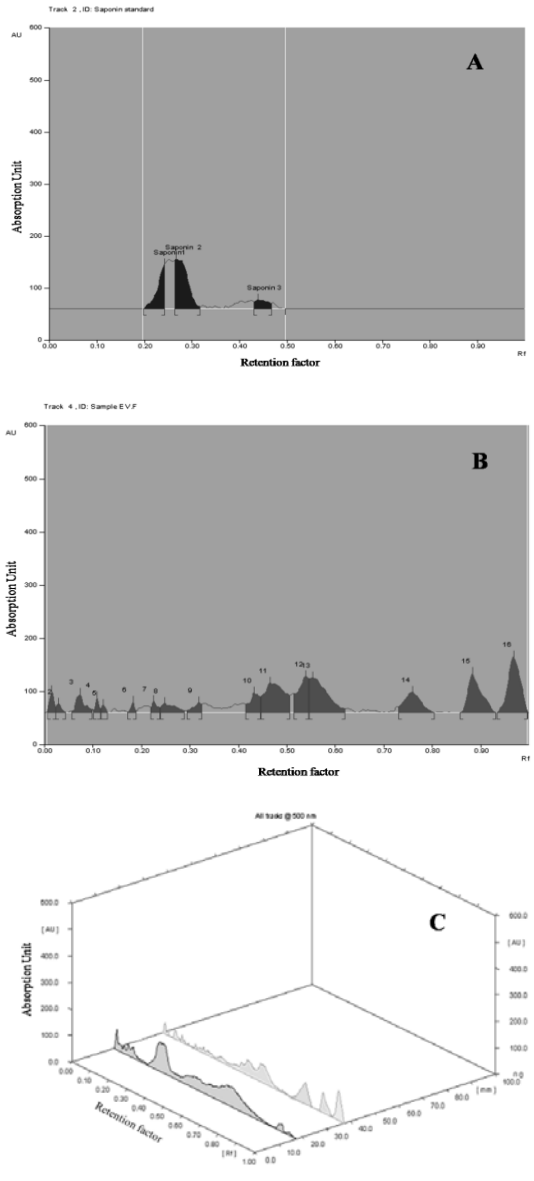


Fig. 8: Densitogram and 3D display for saponin profile of ethanolic extract of *E. variegata* L. and standard. A and B-Densitogram of saponins standard and *E. variegata* L. flowers, C-3D display of saponins standard and *E. variegata* L. flowers

Table 9: Peak table of steroids and unknown compounds in ethanolic extract of *Erythrina variegata* L. flowers

Track	Peak	R_f	Height	Area	Assigned substance
STD	1	0.49	265.3	17577.0	Stigmasterol
Sample EV. F	1	0.02	173.8	1975.9	Unknown
Sample EV. F	2	0.08	390.9	7319.8	Steroid 1
Sample EV. F	3	0.15	89.3	2576.6	Unknown
Sample EV. F	4	0.18	106.8	1626.3	Steroid 2
Sample EV. F	5	0.25	136.9	6688.8	Unknown
Sample EV. F	6	0.46	270.4	12254.0	Steroid 3
Sample EV. F	7	0.48	272.0	8559.4	Unknown
Sample EV. F	8	0.54	218.4	8047.6	Steroid 4
Sample EV. F	9	0.85	19.3	467.7	Steroid 5

EV. F-*Erythrina variegata* L. flowers & STD: Standard

Table 8 represents the Saponin HPTLC profile of ethanolic extract of *Erythrina variegata* L. flower. In this profile, three standards were used, and 4 saponins were detected in the chromatogram of the extract. The R_f values in the reference standard and extract were found to be 0.25, 0.27, 0.44 and 0.23, 0.43, 0.47, 0.76. Fig. 7 and 8 indicates the presence of saponin in *E. variegata* L. flowers which are again displayed in densitogram and 3D display. The band revealed the presence of saponin by its green, yellow, and blue coloured zones at daylight mode after derivatization. Saponins exhibited a wide range of biological activities. On the other hand, saponins also have beneficial pharmacological effects. They are anticholesterolemic due to the formation of a complex with cholesterol in the gastrointestinal tract, thus preventing absorption [22].

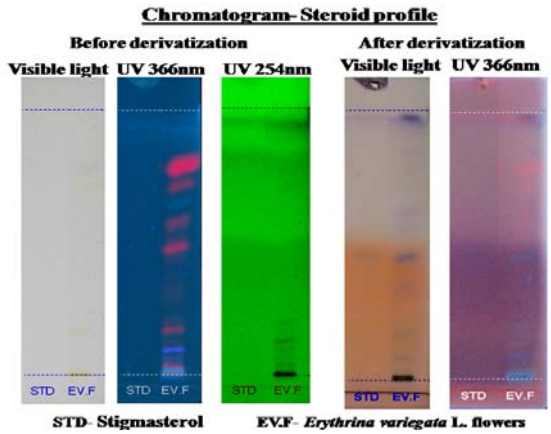
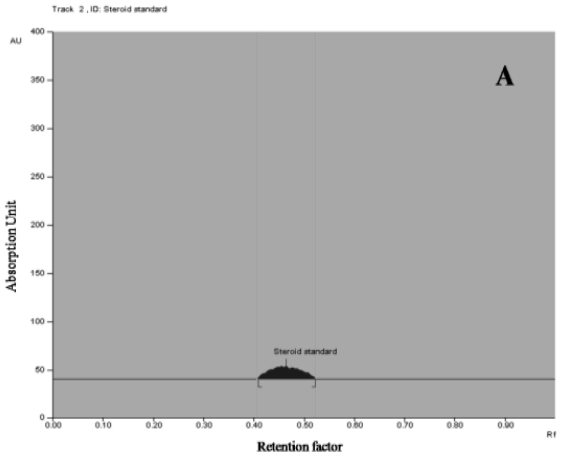
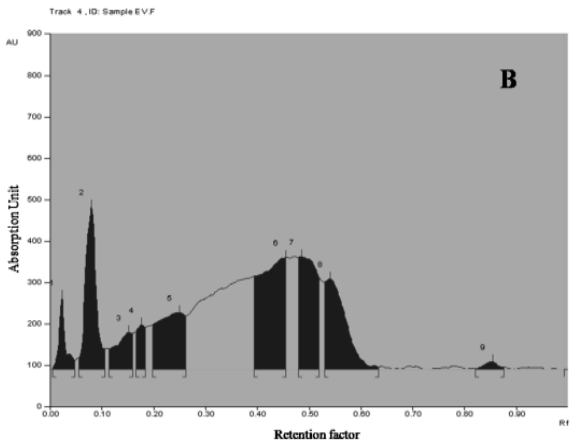


Fig. 9: Chromatograms of ethanolic extract of *Erythrina variegata* L. flowers in HPTLC analysis-Before derivatization under visible light, UV 366 nm and UV 254 nm. After derivatization under visible light and UV 366 nm





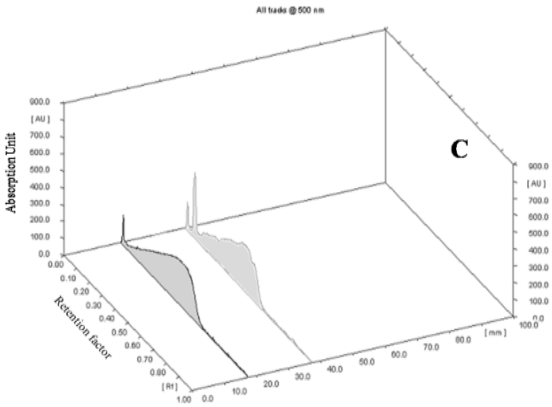


Fig. 10: Densitogram and 3D display for steroid profile ethanolic extract of *E. variegata* L. and standard. A and B-Densitogram of steroid standards and *E. variegata* L. flowers, C-3D display of steroids standard and *E. variegata* L. flowers

The chromatogram of an ethanolic extract of *Erythrina variegata* L. flowers shows the presence of five steroid compounds and four unknown compounds with specific R_f values and peak area (table 5). The TLC plate was visualized at 366 nm and 254 nm, before derivatization and 366 nm after derivatization. Blue and bluish violet coloured zones at the visible light mode are present in the sample observed in the chromatogram after derivatization confirmed the presence of steroid in the given samples.

The dendrogram and 3D display of steroid profile of *E. variegata* L. flowers were shown in fig. 9 and 10. Sterols found in plants are known as phytosterols and over 250 phytosterols and their related compounds have been identified from natural products. Polysterols cannot be synthesized by humans and are thus consumed from the diet [23].

CONCLUSION

In this study, phytoconstituents were identified on the basis of their R_f values. Identified phytoconstituents were confirmed by the visualization at different wavelengths of light and by densitometric analysis of the plate. A conclusion is drawn from this study that the ethanolic extracts of *Erythrina variegata* L. flowers are more effective than the other extracts. The reported data based on the HPTLC fingerprint approach can also be proposed as a quick and reliable analytic model for the pharmacognostic study of plant raw materials used in commercial products.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest

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