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Chemo profiling of hydroalcoholic extract of *Ficus Religiosa* Linn.

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Abstract: Chemo-profiling of herbal drugs represent a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of drugs and their related products. TLC were performed for preliminary identification of constituent in solvent system Toluene: Chloroform: Acetone (40:25:35 v/v/v), gallic acid was used as standard for phenolic compound. Phenolic compound was estimated in hydro-alcoholic extract of *Ficus religiosa* Linn. (Stem bark) by high performance thin layer chromatography (HPTLC). Precoated Silica gel 60 F 254 (E. Merk) TLC plates were used as stationary phase and Toluene: Chloroform: Acetone (40:25:35 v/v/v) was used as mobile phase. Detection and quantification were performed by densitometry at λ 254 nm. The linear range was 200 ng to 600 ng. This HPTLC method was found to reproducible, accurate and precise.

Key Words: Chemo profiling, gallic acid, TLC, HPTLC, *Ficus religiosa* Linn.

Introduction

The medicinal plants generally have multiple bioactive constituents, which are of varied therapeutic and/or nutritional values. The availability and proportion of these bioactive constituents in plant derived medicines and other botanicals depend upon multiple factors such as collection procedure of plant material, geographical location and seasonal variation as well as extraction methods. A change in the proportion of key bioactive constituent can lead to alteration in the ultimate beneficial effects of the herbal preparation. In order to standardize the herbal drugs or functional foods, chemo-profiling of the bioactive compound is important. In view of the industrial application, it is of prime importance to develop chemo-profiling methods which are cost effective, simple and precise [1].

Chromatographic fingerprinting has been in use for a long time for single chemical entity drug substances. Chemical and chromatographic techniques may also be used to aid in identification of herbal medicine or extract and in assessment of their potency and stability [2]. High performance thin layer chromatography (HPTLC) has recently emerged as a preferred analytical tool for fingerprinting and quantification of marker compounds in herbal drugs because of its suitability for high throughput screening sensitivity and reliability in quantification of analytes at nanogram level [3, 4].

Ficus religiosa Linn. is extensively used in traditional systems of medicine like Ayurveda, Unani and Siddha in the form of various formulations. Bark is used in healing ulcers, various skin diseases and scabies and in treatment of diabetes, the root bark is stated to be aphrodisiac. Fruit is laxative and digestive. The fruit powder is also given to enhance fertility and used in dysentery, uterine troubles, ulcers, biliousness, bitter tonic, in blood diseases. All plant parts are acrid, sweetish, cooling and are useful in diseases of blood vagina, uterus, given in leucorrhoea, burning sensation, biliousness and ulcers [5-9]. The reported phytoconstituents of stem bark of *Ficus religiosa* Linn. are phenols, tannins, steroids, alkaloids and flavonoids, β-sitosteryl-d-glucoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one. Stem bark contains three methyl ethers of leucoanthocyanins, delphinindin-3-0-α-L-rhamnoside (1), Pelargonidin-3-0-α-L-rhamnoside (11) and Leucocyanidin-3-0-β-D-galactosyl-cellobioside (111)-along with methyl ether of leucoanthocyanidin isolated from stem bark [10, 11].

Materials and Methods

Collection and Drying

The stem bark of *Ficus religiosa* Linn. was collected from healthy trees of B. N. Institute campus of Udaipur (Raj.) The collected stem barks were dried at room temperature under a well-ventilated shade by distributing them homogenously.

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Authentication
Drug sample was identified by Dr.S.S. Katewa, College of Science, Mohanlal Sukhadia University, Udaipur (Raj). *Ficus religiosa* Linn. was authentified from Botanical Survey of India (BSI), Jodhpur (Raj), the letter no. BSI/AZRC/I.12012/Tech.393.

Preparation of Extract
The dried powdered stem bark of *Ficus religiosa* Linn. was weighed and extracted with an appropriate solvent (Ethanol: Water-60:40) in a soxhlet apparatus. The extract was then filtered and dried in vacuum evaporator at 38°C [12].

Preliminary Qualitative Tests
The hydro-alcoholic extract of stem bark of *Ficus religiosa* Linn. was subjected to preliminary qualitative phytochemical investigation [13, 14].

Thin Layer Chromatography (TLC)
The preliminary phytochemical investigation revealed the presence of tannins, flavonoids and phenolic compounds. The extracts of stem bark of *Ficus religiosa* Linn. was subjected to thin layer chromatography to detect the various constituents present in it.

Adsorbent : Silica gel GF 254 (activated)
Thickness : 0.2 mm
Plate size : 12 x 18 cm
Activation temp : 110°C for 1hr
Volume of spot : 20µl
Solvent system : Toluene:Chloroform:Acetone (40:25:35 v/v/v)

The spots were observed in UV chamber [15, 16].

High Performance Thin Layer Chromatography (HPTLC)
Preparation of Standard Solution: The reference standard solution of galic acid was prepared in methanol in concentration range of 100 ng to 700 ng.

Chromatographic Conditions: The following chromatographic conditions were used to quantify the gallic acid:

Stationary phase : Silica gel 60 F 254 (E. Merck) precoated TLC plates
Mobile Phase : Toluene: Chloroform: Acetone (40:25:35 v/v/v)
Sample volume : 2 µl
Temperature : 60°C
Migration Distance : 70 mm
Detection wavelength : 254nm

Linearity was performed by applying standard solution at different concentrations ranging from 100 ng to 700 ng on 20 × 20 cm HPTLC plates, precoated with silica gel 60 F 254 (E. Merck) in the form of sharp 8 mm bands. The plates were developed in a solvent system of Toluene: Chloroform: Acetone (40:25:35 v/v/v), up to a distance of 70mm, at 60°C. The detector response for galic acid was measured for each band at wavelength of 254 nm, using Camag TLC Scanner and win CAT software. The peak areas of Gallic acid were recorded for each concentration. The linearity curve of Gallic acid was obtained by plotting a graph of peak area of Gallic acid *vs* applied concentrations of Gallic acid (ng).

Result and Discussion

Preliminary Phytochemical Analysis
Phytochemical screening of hydro-alcoholic extract of *Ficus religiosa* Linn., showed the presence of various chemical constituents mainly alkaloids, flavonoids, tannins, phenolic compounds and steroids.

Table 1: Phytochemical screening of stem bark extracts of *Ficus religiosa* Linn.

Chemical Test		Hydro-alcoholic extract (Ethanol 60%)
Alkaloids	Dragondoff's Test	+
	Wagner's Test	+
	Mayer's Test	+
	Hager's Test	+
Glycosides	General Test	+
	Borntrager Test	+
	Killer Killiani Test	-
	Legal's Test	+
Flavonoids	Lead Acetate Test	+
	Shinoda Test	+
Steroids	Salkowaski Test	+
Tannis & Phenolic compounds	$FeCl_3$ Test	+
Amino Acids	Ninhydrin Test	-
Carbohydrates	Molisch's Test	+
Reducing Sugar	Fehling's Test	+
	Benedict's Test	+
Proteins	Ethanol Test	-
Saponins	Foam Test	-
Fixed Oils & Fats		-
Volatile Oil		+

Phytochemical screening of Hydroalcoholic extract of *Ficus religiosa* Linn. prepared by soxhlet, extraction method showed the presence of various chemical constituents

mainly alkaloids, flavonoids, tannins and phenolics and steroids. The results obtained were comparable and satisfied the standard literature. The reported phytoconstituents of stem bark of *Ficus religiosa* Linn. are phenols, tannins, steroids, alkaloids and flavonoids, β-sitosteryl-d-glucoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one [17]. The phytochemistry of this plant shows that in the bark (-) epicatechin, procyanidin P2, 11'-deoxyprocyanidin B, (+) catechin, (24, £)-24-methyl-cholesta-5-en-3p-ol (22 E, 21£)-24-ethylcholesta-5, 22 dien-33-ol, (24 £)-24-ethylcholesta-54-en-3p-ol, leucopelargonidin-3-O-p-D-glucoside, leuco-pelargonidin and leucocyanidin [18-20].

Thin Layer Chromatography

Thin layer chromatography of extract of *Ficus religiosa* stem bark revealed violet green spot under UV (R_f = 0.52) which are almost comparable to that of standard gallic acid (R_f = 0.52) and showed the presence of phenolic content in plant extract.

High Performance Thin Layer Chromatography (HPTLC) Ficus religiosa Linn.

Substance: Gallic acid @ 254nm
Regression via height: Polynomial
Y= -218.7+ 2.458 * X + -0.003073 * X2
r = 0.99999 sdv = 0.00
Regression via area: Polynomial
Y= -3721+ 35.7 * X + -0.04513 * X2
r = 0.99999 sdv = 0.00

Table 2: HPTLC profile of standard gallic acid

S. No.	R_f Value	Concentration	Peak Height	Peak Area
1.	0.54	200 ng	150.11	1614.40
2.	0.56	400 ng	273.07	3339.71
3.	0.56	600 ng	150.20	1454.76

Table 3: HPTLC profile of hydro-alcoholic extract of *Ficus religiosa* Linn.

S. No.	R_f Value	Peak Height	Peak Area	Quantitative Yield
1.	0.55	259.21	3247.10	350.06 ng
2.	0.54	223.63	2637.05	270.72 ng
3.	0.56	228.92	2639.96	270.98 ng

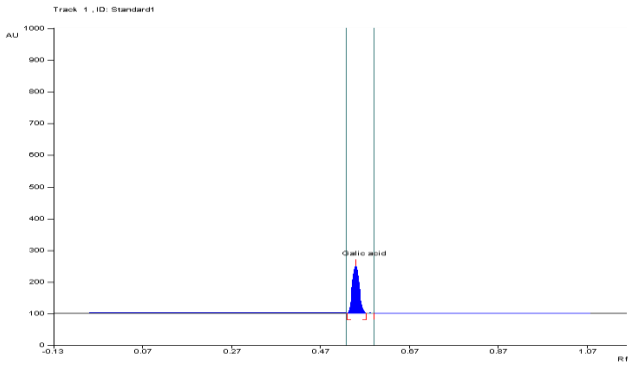

Figure 1: HPTLC chromatogram of gallic acid (standard)

The method utilizes silica gel 60F 254 HPTLC plates as stationary phase and Toluene: Chloroform: Acetone (40: 25: 35 v/v/v) as mobile phase which gives good separation of gallic acid. The identity of the band of gallic acid in the sample extract was confirmed by overlaying the UV absorption spectra of samples with that of reference standard which showed λmax at 254nm.

Phytochemical investigations (qualitative chemical analysis, TLC and HPTLC) were carried out with hydro-alcoholic extract of *Ficus religiosa* Linn. Qualitative chemical analysis and TLC determination showed the presence of several phyto-constituents like phenolic compounds, flavonoids, steroids, tannins, carbohydrates and the presence of phenolic compound was confirmed by HPTLC.

Conclusion

In conclusion, the proposed HPTLC method was found to be precise, specific, accurate and robust and can be used for identification and quantitative determination of herbal extract and its formulations. HPTLC method is especially suitable for the fingerprinting and high throughput analysis of botanical samples and herbal formulations.

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