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HPTLC CHEMO-PROFILING OF RAW MATERIALS AND FORMULATIONS OF SUFOOF-E-BARS: A UNANI FORMULATION FOR VITILIGO

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ABSTRACT

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This traditional knowledge forms the recognized indigenous systems of medicine and exist in the forms of Ayurveda, Unani and Siddha. Sufoof-e-Bars is a traditional Unani formulation used for clinical treatment of Vitiligo. High-Performance Thin-layer Chromatography has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. Therefore in the present study, for the first time HPTLC chemo-profiling was developed for raw materials and formulations of Sufoof-e-Bars. Thus, the HPTLC chemo-profiling of the botanically authenticated raw materials and formulations of Sufoof-e-Bars will serve as primary reference for quality control and quality assurance.

KEYWORDS*:* Sufoof-e-Bars, Chemo-profiling and HPTLC.

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from several medical texts and manuscripts. This traditional knowledge forms the recognized indigenous systems of medicine and exist in the forms of Ayurveda, Unani, Siddha and Swa-riga (Tibetan) systems of medicine (Perumal and Gopala, 2007).

Despite the large number of drugs derived from total synthesis, plant-derived natural products still contribute to the overall total number of New Chemical Entities (NCE). Several reviews on drug discovery and development from natural sources (plants, marine fauna and microbes) have been published in last few decades (Butler, 2005; Chin *et. al.*, 2001). Currently, one fourth of all prescribed pharmaceuticals in industrialized countries contain compounds that are directly or indirectly derived from plants. Furthermore, 11% of the 252 drugs considered as basic and essential by WHO are exclusively derived from flowering plants (Rates, 2001).

The pharmacological action of crude drug is determined by the nature of its constituents. Thus, the plants species may be consider as a biosynthetic laboratory not only for chemical compounds like carbohydrates, proteins and fats (primary metabolites) that are utilized as food by humans and animals, but also for a magnitude of compounds like alkaloids, terpenoids, flavonoids, glycoside (Secondary metabolites) etc. which exert a definite physiological effects.

These chemical compounds are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials are used as such in their crude form or they may be extracted with suitable solvent to take out the desired components and the resulting principle compound being employed as therapeutic agent (Harborne, 2007; Mukharjee, 2002).

An intact botanical raw material can be taxonomically (macroscopically and microscopically) defined and proved useful by a long tradition of use. That says nothing about its chemical composition. This composition can be affected by age, environmental factors such as mineral nutrition and stress, geographical origin and postharvest handling and storage. The botanical identity must be matched with a chemical profile. Unfortunately, in most case there is no simple link between that profile and the efficacy of the drug as a measure of quality (Anonymous, 2009). To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there has been emphasis on standardization of medicinal plants of therapeutic potential (Patil *et. al.*, 2013).

For wider, more reliable and scientific application of traditional knowledge of medicines, researchers are continuously subjecting it to a variety of tests through field, laboratory or clinical research (Jain, 2004). Therefore, the present challenge for Unani scholar is to validate the claims applying scientific and analytical methodologies (Ahmed, 2010). Analytical

separation techniques, like High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) and Mass Spectrometry (MS) are among the most popular methods of choice used for quality control of raw material and finished herbal product (Ram *et. al.,* 2011). There are many chromatographic methods available; HPTLC has become the most potent tool for quality control of herbal product. The especial advantages of HPTLC are its sensitivity, speed, simplicity, reliability and versatility. Sensitivity allows separation of less than nanogram amount of raw materials (Eike and Anne, 2006).

HPTLC provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drug and for detecting adulteration and substitutions. A photographic HPTLC atlas fulfils the same function and purpose as a catalogue of spectra. The identity and non identity of a official drug can be established by comparison with the chromatogram of the 'Standard drug'. Unknown commercial drugs can be more easily identify by comparison with the visual records. The photographic drug atlas is an aid to the routine identification and purity testing of drug. In drug control laboratories photographic reproduction of thin layer separation has an advantage over mere graphic representation (Patil *et. al.*, 2013).

Vitiligo is one of the commonest skin disorder characterized by the appearance of completely de-pigmented milky white macules of varying sizes and shapes. Besides loss of color there are no other structural changes (Mitchell, 1996). In India, complex social stigmas arising out of various erroneous notions about the blemishes are attached to the disease. Vitiligo certainly inflicts tremendous psycho-sociological stress on patients.

Sufoof-e-Bars (SEB) is a traditional Unani formulation mentioned in National Formulary of Unani Medicine (1995) for clinical treatment of Vitiligo (Anonymous, 1995). However, during ethno-medicinal survey it has been observed that few traditional Unani practitioners (*Hakims*) modify this formulation for the better results. Therefore, in the present work those two modified SEB formulations are also included for chemo-profiling.

Although, SEB formulation is clinically used for the treatment of Vitiligo from ancient time, a comprehensive literature search revealed the lack of report on HPTLC chemo-profiling of secondary metabolites present in raw materials and formulations of SEB. Therefore, in the present study, for the first time HPTLC chemo-profiling of secondary metabolites for raw materials and formulations of SEB was developed. The data obtained in the present work

could be useful in proper identification and authentication of the raw materials and formulations which is a pre requisite for recognition of traditional medicines at global level.

MATERIALS AND METHODS

Materials and Reagents

SEB is prepared using multiple botanical ingredients *viz.* seeds of *Psoralea corylifolia* Linn. (*Babchi*), seeds of *Cassia absus* Linn. (*Chaksu*), dried fruits of *Ficus carica* Linn. (*Anjeer khushk*) and seeds of *Cassia tora* Linn. (*Tukhm-e-panwar*) as mentioned in National Formulary of Unani Medicine (1995). The raw materials of SEB were procured from Unani drug shop, Bhiwandi, Thane, Maharashtra, India and authenticated from Agharkar Research Institute, Pune, M.S., India. All these raw materials were observed carefully and foreign matters were removed. The materials were kept in oven at 40±2 ^{o}C for drying. The dried raw materials were finely powdered separately, passed through 355/180 # sieve and stored in air tight containers. The raw materials were mixed in equal part to get uniformly blended SEB F1 (Anonymous, 1995) and the first modified SEB (F2) was prepared with slight change in ratio of national formulary's formulation and addition of *Ipomea hederacea* Jacq. (Kala dana), and the second modified formulation (F3) have addition of Red auichour (Gairoo). The Merck made analytical grade chemicals were used for extraction and mobile phase preparation.

High Performance Thin Layer Chromatography (HPTLC) Analysis

HPTLC chemo-profiling of raw materials and formulations of SEB were carried out for secondary metabolites *Viz.* anthracene derivatives, arbutin derivatives, alkaloids, bitter drug, cardiac glycosides, coumarin derivatives, essential oils, liganans, pungent testing principles, saponins, triterpenes and valeportriates as per the standard methodology described by Wagner and Bladt, 1984.

Analysis work was carried out on HPTLC equipment, CAMAG made (Muttenz, Switzerland) and consisted of Linomat-V sample applicator fitted with a 100 µL syringe (Hamilton, Switzerland), Camag TLC Scanner 3, Camag TLC visualizer and WinCATS software. Analysis was performed by using TLC precoated silica gel 60 F254 aluminium plates (15 × 10 cm) with respective mobile phases. Mobile phase linear ascending development was carried out in a twin-trough glass chamber saturated with mobile phase. The optimized chamber saturation time for the mobile phase was 10-20 mints at room temperature (25± 2°C) at a relative humidity of 50 ± 5%. The TLC plates were developed up to the distance of 8 cm.

After development densitometric scanning was performed at 254, 366 nm and visible light. The slit dimensions were 6 mm × 0.45 mm, and the scanning speed was 20 mm s^{-1}.

Sample extraction

Powdered raw materials and formulations were extracted in different solvents using methodology described by Wagner and Bladt, 1984. All the samples were filtered through a Whatman filter paper before HPTLC analysis.

Detection of bands

After development, plates were dried at room temperature, dervatized with freshly prepared derivatizing reagents in a derivatisation chamber for 30 seconds and dried at room temperature. After drying plates were heated in oven at 110^{o}C for 10 mins. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and the images were captured under 254, 366 nm and visible light as required.

RESULTS AND DISCUSSION

Recognition of the biological properties of myriad natural products has fuelled the current focus of this field, namely, the search for the new drug, antibiotics, insecticides and herbicides. Based on their biosynthetic origin, plant natural products can be divided into three major groups: the terpinoids, the alkaloids and the phenolic compounds. All terpenoids, including both primary metabolites and more than 25,000 secondary compounds are derived from the five carbon precursor isopentenyl diphosphate (IPP) (Rodney, et. al., 2002).

HPTLC has become a routine analytical technique due to its advantages of reliability in quantitation of analytes at micro and even in nanogram levels and cost effectiveness (Sethi, 1992). It has proved a very useful technique because of its low operating cost, high sample through output and need for minimum sample clean-up.

HPTLC chemo-profile for secondary metabolites in raw materials and formulations of SEB has been done in the present work. The samples were extracted and analyzed using HPTLC. The results are a specific sequence of peak or zones due to known or unknown component of the extracts. The fingerprint of the botanically authenticated raw materials serves as primary reference against which unknown raw material can be characterized (EIke and Anne, 2006). The studies revealed that the solvent systems developed and the specific dervatizing reagents

used gave well resolved bands for secondary metabolites present in raw materials and formulations of SEB (Table-1).

Table1: Solvent systems and spray reagents for secondary metabolites.

Sr. No	Phytoconstituents	Solvent systems	Spray reagents	Inference
1.	Alkaloids	Toluene: Ethyl acetate: Diethyl amine (70:20:10)	Dragendorff reagent	The alkaloids appear as brown zones immediately after spraying.
2.	Anthracene derivatives	Ethyl acetate: Methanol: Water (81:11:8)	Potassium hydroxide reagent	The anthracene derivatives appear as yellow or red-brown fluorescence at 366 nm.
3.	Arbutin derivatives	Ethyl acetate: Methanol: Water (100:13.5:10)	Gibb's reagent	Arbutin becomes blue violet at visible light.
4.	Bitter Principles	Ethyl acetate: Methanol: Water (77:15:8)	Anisaldehyde – Sulphuric acid reagent	Red-violet, brown, blue-green, blue, grey spots shows presence of bitter principles at visible light.
5.	Cardiac Glycosides	Ethyl acetate: Methanol: Water (81:11:8)	Sulphuric acid reagent	Yellow brown and blue zones shows presence of cardiac glycosides in visible light after derivatisation.
6.	Coumarin derivatives	Toluene: Ether (1:1 saturated with 10% acetic acid) lower phase	Potassium hydroxide reagent	In 366 nm coumarins appear as blue, blue green and yellow fluorescence.
7.	Essential Oils	Toluene: Ethyl acetate (93:7)	Anisaldehyde-Sulphuric acid reagent	Evaluation with anisaldehyde shows strong blue, red, green and brown coloration for essential oils.
8.	Lignans	Toluene: Ethyl acetate (70:30)	Sulphuric acid reagent	Lignans gives blue

				fluorescence at 366 nm.
9.	Pungent -Tasting Principles	Toluene: Ethyl acetate (70:30)	Vanillin – Sulphuric acid reagent	Pungent tasting principles appear as lemon yellow and blue to violet after derivatisation.
10.	Saponins	Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8)	Vanillin – Sulphuric acid reagent	Saponin forms mainly Blue, Blue-violet and yellow brown zones at visible light.
11.	Triterpenes	Ethyl acetate: Glacial acetic acid: Formic acid: Water (90:3:3:2)	Anisaldehyde-Sulphuric acid reagent	Triterpenes appears blue-violet dumbbell shape quenching under 366 nm.
12.	Valepotraites	Toluene: Ethyl acetate (75:25)	Anisaldehyde-Sulphuric acid reagent	A violet and blue zone shows presence of valepotriates.

The results on presence and absence of secondary metabolites in raw materials and formulations are given in Table-2 where as the Rf values of the secondary metabolites in raw materials and formulations are tabulated in Tables 3-5. The chromatograms are presented in Fig-1 and 2.

Table-2. Results of HPTLC chemo-profiling of raw materials and formulations of SEB.

Sr.No	Phytoconstituents	Raw materials and formulations							
		Pc	Ca	Fc	Ct	Ih	F1	F2	F3
1.	Alkaloids	+	-	-	+	+	+	+	+
2.	Anthracene derivatives	-	-	-	+	-	+	+	+
3.	Arbutin derivatives	+	-	-	+	+	+	+	+
4.	Bitter Principles	+	+	+	+	+	+	+	+
5.	Cardiac Glycosides	+	+	+	+	+	+	+	+
6.	Coumarin derivatives	+	+	-	+	+	+	+	+
7.	Essential Oils	+	+	+	+	+	+	+	+
8.	Lignans	+	+	+	+	+	+	+	+
9.	Pungent – Tasting Principles	+	+	+	+	+	+	+	+
10.	Saponins	+	+	+	+	+	+	+	+
11.	Triterpenes	+	-	-	+	+	+	+	+
12.	Valepotraites	+	+	+	+	+	+	+	+

Key words: *Pc- Psoralea corylifolia Linn., Ca- Cassia absus Linn., Fc- Ficus carica Linn., Ct- Cassia tora Linn., Ih- Ipomea hederacea Jacq., F1- SEB formulation as per the National*

Formulary of Unani Medicine, F2- SEB modified formulation 1st, F3- SEB modified formulation 2nd ,(+) - Present and(-) -Not Detected.

Table – 3 Retention factors of secondary metabolites present in raw materials and formulations of SEB.

Name	Rf
	Alkaloids
Pc	0.05, 0.09, 0.12, 0.20, 0.28, 0.38, 0.59
Ct	0.17, 0.22
Ih	0.15
F1	0.38, 0.59
F2	0.15, 0.38,0.59
F3	0.38, 0.59
	Anthracene derivatives
Pc	0.15, 0.70
Ca	0.15
Ct	0.07,0.41
F1	0.40, 0.07, 0.70
F2	0.40, 0.70
F3	0.07, 0.40,0.70
	Arbutin derivatives
Pc	0.44, 0.51,0.57, 0.62
Ct	0.14, 0.40
Ih	0.56
F1	0.14, .40
F2	0.14, 0.40
F3	0.14, 0.40, 0.51,0.56
	Bitter Principles
Pc	0.05, 0.11, 0.36, 0.55, 0.67, o.87
Ca	0.11, 0.16, 0.20,0.56,0.66,0.85
Ct	0.09,0.61, 0.20, 0.56, 0.66, 0.85
Ih	0.12, 0.20,0.28,0.34,0.43,0.56,0.59,0.66,0.69,0.79
F1	0.05,0.18,0.30,0.56,0.66,0.87
F2	0.05, 0.09, 0.21,0.30, 0.56, 0.61, 0.68, 0.75,0.87
F3	0.05,0.09,0.21,0.31,0.61,0.68,0.75,0.87
	Cardiac glycosides
Pc	0.27, 0.31, 0.4, 0.55, 0.67
Ca	0.09
Fc	0.11
Ct	0.07,0.16,0.24,0.43,0.68
Ih	0.05,0.11,0.32,0.40,0.43, 0.47,0.53, 0.61, 0.67
F1	0.05, 0.12,0.23, 0.45, 0.55,0.68
F2	0.05,0.09,0.21,0.40, 0.47, 0.53, 0.57, 0.69
F3	0.09, 0.11, 0.19, 0.24, 0.28, 0.45, 0.55, 0.61,0.68

***Key words**: Pc- Psoralea corylifolia Linn., Ca- Cassia absus Linn., Fc- Ficus carica Linn., Ct- Cassia tora Linn., Ih- Ipomea hederacea Jacq., F1-SEB formulation as per the National Formulary of Unani Medicine, F2- SEB modified formulation 1st and F3- SEB modified formulation 2nd .*

Table – 4 Retention factors of secondary metabolites present in raw materials and formulations of SEB.

Name	Rf
	Coumarin derivatives
Pc	0.07,0.10, 0.18, 0.23, 0.27, 0.30, 0.35, 0.39, 0.59
Ca	0.12, 0.20, 0.23, 0.28, 0.33, 0.36, 0.39, 0.42
Fc	0.44
Ct	0.06, 0.28, 0.43, 0.65, 0.70,0.77, 0.85
Ih	0.20,0.42,0.70
F1	0.07,0.18, 0.28,0.40,0.44,0.59, 0.68, 0.77, 0.84
F2	0.07, 0.18, 0.28, 0.40, 0.44, 0.59, 0.69, 0.77, 0.84
F3	0.07, 0.18, 0.28, 0.40, 0.44, 0.59, 0.69, 0.77, 0.84
	Essential oils
Pc	0.07, 0.11, 0.17, 0.23, 0.27, 0.30, 0.43, 0.53, 0.64, 0.77
Ca	0.06, 0.18, 0.26, 0.40, 0.51, 0.67, 0.76
Fc	0.19, 0.31, 0.40, 0.64, 0.78
Ct	0.19, 0.24, 0.40, 0.55, 0.65, 0.77
Ih	0.14, 0.19, 0.24, 0.40, 0.55, 0.65, 0.77
F1	0.06, 0.07, 0.11, 0.19, 0.23, 0.26, 0.30, 0.53, 0.58, 0.73, 0.87, 0.92
F2	0.06, 0.07, 0.11, 0.19, 0.23, 0.26, 0.30, 0.53, 0.60, 0.68, 0.74, 0.85, 0.92
F3	0.06, 0.07, 0.11, 0.19, 0.23, 0.26, 0.30, 0.53, 0.58, 0.73, 0.86, 0.92
	Lignans
Pc	0.07,0.10, 0.18, 0.23, 0.27, 0.30, 0.35, 0.39, 0.59
Ca	0.12, 0.20, 0.23, 0.28, 0.33, 0.36, 0.39, 0.42
Fc	0.44
Ct	0.06, 0.28, 0.43, 0.65, 0.70,0.77, 0.85
Ih	0.20,0.42,0.70
F1	0.07,0.18, 0.28,0.40,0.44,0.59, 0.68, 0.77, 0.84
F2	0.07, 0.18, 0.28, 0.40, 0.44, 0.59, 0.69, 0.77, 0.84
F3	0.07, 0.18, 0.28, 0.40, 0.44, 0.59, 0.69, 0.77, 0.84
	Pungent tasting principles
Pc	0.30, 0.35, 0.41, 0.46, 0.86
Ca	0.33, 0.49, 0.67, 0.86
Fc	0.46, 0.59, 0.79,0.86
Ct	0.45, 0.50, 0.66, 0.87
Ih	0.30, 0.49, 0.67, 0.87
F1	0.30, 0.35, 0.41, 0.46, 0.86
F2	0.30, 0.35, 0.41, 0.46, 0.86
F3	0.30, 0.35, 0.41, 0.46, 0.86

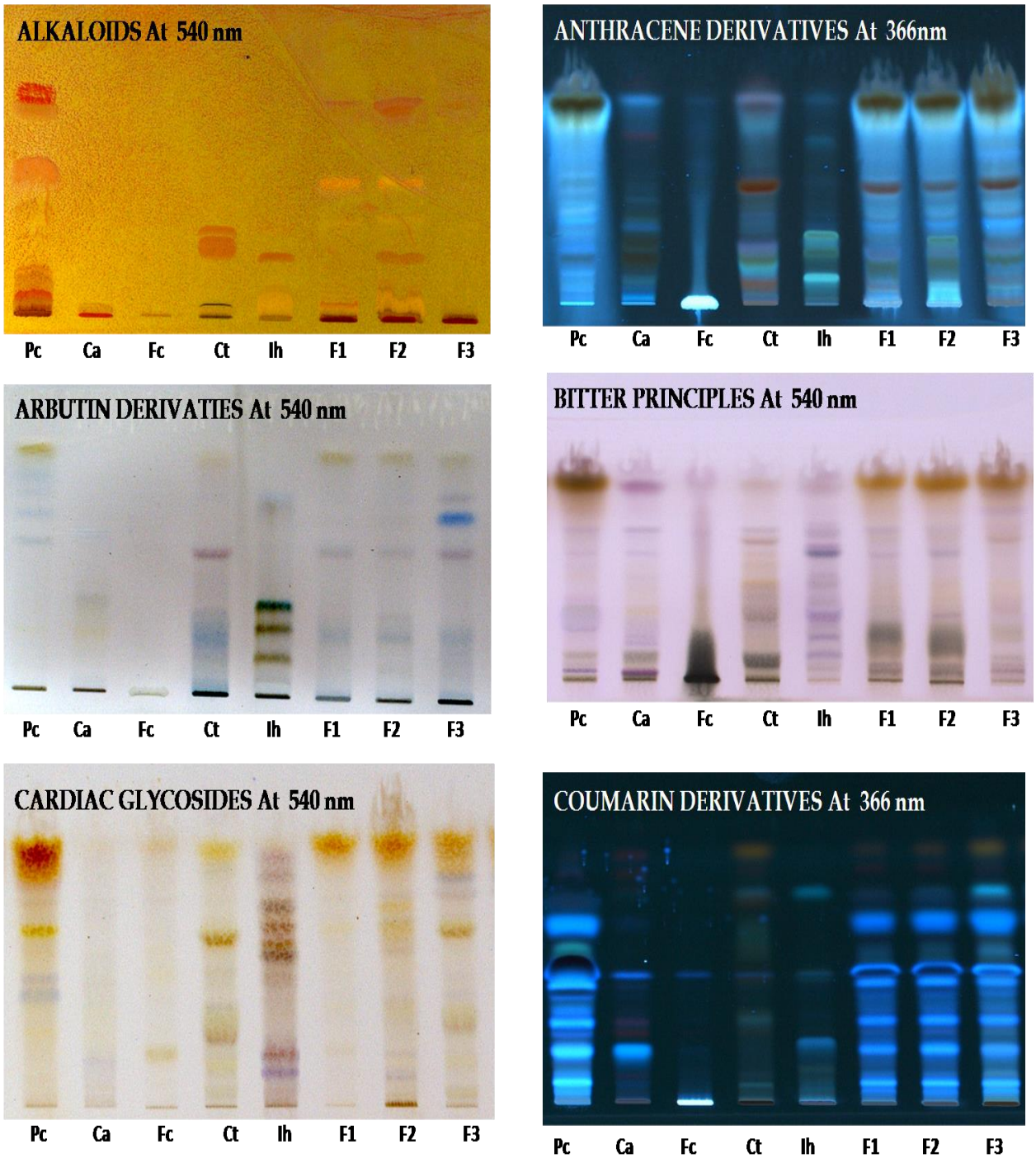
***Key words**: Pc- Psoralea corylifolia Linn., Ca- Cassia absus Linn., Fc- Ficus carica Linn., Ct- Cassia tora Linn., Ih- Ipomea hederacea Jacq., F1-SEB formulation as per the National Formulary of Unani Medicine, F2- SEB modified formulation 1st and F3- SEB modified formulation 2nd .*

Table – 5 Retention factors of secondary metabolites present in raw materials and formulations of SEB.

Name	Rf
	Saponin
Pc	0.05, 0.13, 0.24, 0.28, 0.32, 0.38, 0.48, 0.83
Ca	0.13, 0.27, 0.34, 0.40, 0.45, 0.58
Ct	0.21, 0.33, 0.44, 0.56, 0.80
Ih	0.25, 0.53, 0.61, 0.69
F1	0.24, o,36, 0.44, 0.56, 0.83
F2	0.05, 0.25, 0.36, 0.43, 0.48, 0.83
F3	0.13, 0.24, 0.33, 0.48, 0.58, 0.83
	Triterpenes
Pc	0.85
Ct	0.27, 0.50, 0.74, 0.86
Ih	0.71, 0.85
F1	0.85
F2	0.71, 0.85
F3	0.85
	Valeportriates
Pc	0.44, 0.86
Ca	0.84
Fc	0.64, 0.78, 0.84
Ct	o.84
Ih	0.84 0.51, o.84
F1	0.44, 0.86
F2	0.44, 0.86
F3	0.44, 0.86

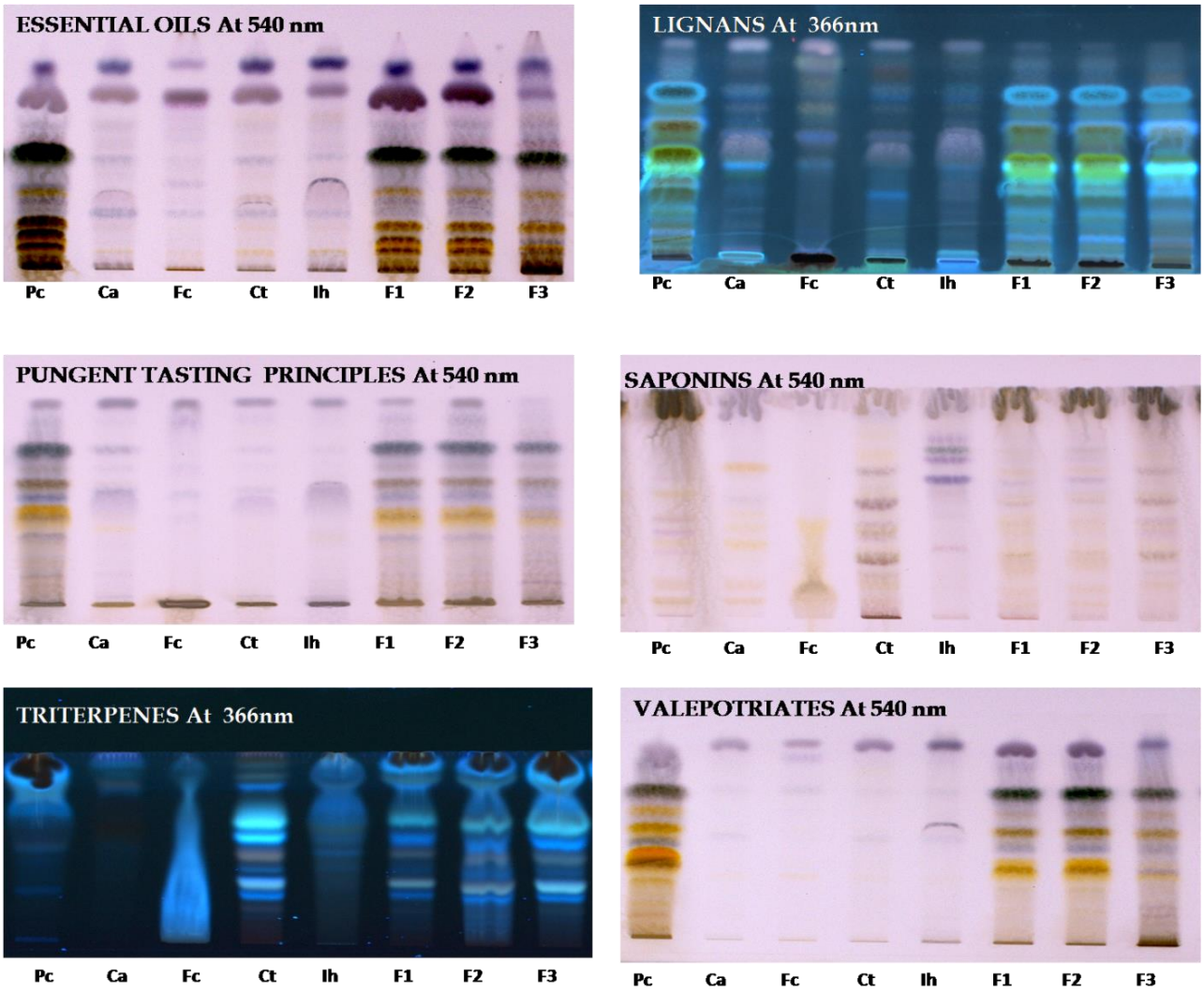
Key words: *Pc- Psoralea corylifolia Linn., Ca- Cassia absus Linn., Fc- Ficus carica Linn., Ct- Cassia tora Linn., Ih- Ipomea hederacea Jacq., F1-SEB formulation as per the National Formulary of Unani Medicine, F2- SEB modified formulation 1st and F3- SEB modified formulation 2nd.*

Fig-1 : HPTLC chromatograms of secondary metabolites present in raw materials and formulations of SEB.



***Key words**: Pc- Psoralea corylifolia Linn., Ca- Cassia absus Linn., Fc- Ficus carica Linn., Ct- Cassia tora Linn., Ih- Ipomea hederacea Jacq., F1-SEB formulation as per the National Formulary of Unani Medicine, F2- SEB modified formulation 1st and F3- SEB modified formulation 2nd .*

Fig-2 : HPTLC chromatograms of secondary metabolites present in raw materials and formulations of SEB.



***Key words**: Pc- Psoralea corylifolia Linn., Ca- Cassia absus Linn., Fc- Ficus carica Linn., Ct- Cassia tora Linn., Ih- Ipomea hederacea Jacq., F1-SEB formulation as per the National Formulary of Unani Medicine, F2- SEB modified formulation 1st and F3- SEB modified formulation 2nd .*

CONCLUSION

There are over 400 different tribal and other ethnic groups in India which constitute about 7.5 % of India's population. Tribal, rural and primitive societies have discovered solution for treatment of diseases to almost all their needs and problems from the natural resources around them. Hence, in recent years, ethno-medicinal studies received much attention as this brings to light the numerous little known and unknown medicinal virtues especially of plant origin

which needs evaluation on modern scientific lines such as phytochemical analysis using HPTLC.

The results obtained from the present studies indicated that the HPTLC profile for several secondary metabolites extracted from the raw materials and formulations of SEB may play an important role in identification and evaluation of quality of raw materials and formulations of this medicinally important Unani remedy for Vitiligo.

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