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Pharmacognostic evaluation and fingerprint profile of raw materials and formulation of Sufoof-e-bars: A remedy for vitiligo

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Abstract
Vitiligo is one of the commonest skin disorders characterized by the appearance of completely de-pigmented milky white macules of varying sizes and shapes which certainly inflicts tremendous psycho-sociological stress on patients. Sufoof-e-Bars is a traditional Unani formulation widely used for clinical treatment of Vitiligo. The present investigation was therefore undertaken to determine the requisite pharmacognostic standards, phytochemical analysis, fluorescence analysis, heavy metal detection and HPTLC fingerprint profile of raw materials and Sufoof-e-Bars. The results obtained for physico-chemical constants were within the acceptable pharmacopeial limits. The preliminary phytochemical analysis of raw materials showed the presence of Alkaloids, Glycosides, Steroids, Tannins etc. The HPTLC fingerprint profile developed for the separation of phytoconstituents is unique to raw materials and formulation of Sufoof-e-Bars. Thus, the scientifically generated data from the current studies will help to overcome batch to batch variation and will also project this formulation in global market in a proper prospective.

Keywords: Sufoof-e-Bars, pharmacognosy, phytochemical analysis, HPTLC fingerprint profile

Introduction
History records the fact that almost every civilization around the world has observed the contribution of herbs and their use as food and medicines. The oldest prescriptions display numerous ancient pharmaceutical and medicinal uses of botanicals and foods, documented on Babylonian clay tablets and the hieratic writing of ancient Egypt on papyrus [1].
Medicinal plants play a pivotal role for the development of new drugs. In the last century, approximately 121 pharmaceutical products were formulated based on the traditional knowledge derived from various sources [2]. Since ancient time use of medicinal plants by Indian, Chinese, Egyptian, Greek and Roman civilization is well documented. The various traditional systems of Medicines such as Siddha, Ayurveda and Unani, use several plant species to treat different ailments [3]. Now-a-days traditional medicines and complementary and alternative medicines are attracting more and more attention with the context of health care provision and health sector reforms.
Under the parasol of traditional medicine systems, the Unani system of medicine is also gaining global acceptance due to the amazing clinical efficiency of the formulations [4]. *Tibb-e-Unani* (Unani medicine) claims to possess many safe and effective single drugs and compound formulations of herbal, animal and metal origin which are used to cure a wide range of diseases.
Although such Unani medicines have been used since ancient times, there is negligible documented evidence regarding their standardization and quality control[4]. Quality control and quality assurance of such compound traditional formulations relies upon good manufacturing practices with adequate batch to batch analysis and standardized method of preparation [5]. These traditional formulations should full-fill tests for identity, potency, purity, safety and efficacy as per WHO guidelines [6, 7].
Vitiligo is one of the common skin disorders characterized by loss of normal melanin pigments in the skin which produces white patches of varying sizes and shapes [8]. Vitiligo is an acquired, idiopathic, and worldwide common depigmentation disorder with an estimated prevalence from 0.1 to 8%. Vitiligo is a chronic autoimmune depigmenting skin disorder characterized by patches of the skin losing functional melanocytes [9].

The Unani physicians depicted Bars in a comprehensive and systemic manner as repugnant and odious ailment which is of great antiquity. The first record of Bars was found in the history of Iranian Medicine, about 2200 BC during the period of Aushoorgan [10] followed by in "Ebers Papyrus" (1550 BC) mentioning two types of diseases which affect the color of the skin, one of them was probably Vitiligo [11].

In India, Unani system of traditional medicine is acquiring more support in primary healthcare and various clinical trials have been undertaken in the light of Unani concepts [12]. Sufoof-e-Bars (SEB) is a traditional Unani medicine commonly used for clinical treatment of vitiligo. Though this Unani powdered formulation enjoys great reputation, its standardization and quality control parameters are not well defined. In the present study, an attempt has been made to investigate pharmacopeial standards and to develop HPTLC fingerprint profile of raw materials and SEB formulation.

Materials and Methods

Raw Material and Preparation of Formulation

SEB is prepared using raw materials *viz.* seeds of *Cullen Corylifolium* (L.) Medik. [Syn. = *Psoralea corylifolia* Linn. (*Babchi*)], seeds of *Chamaecrista absus* (L.) H. S. Irwin & Barneby. [Syn = *Cassia absus* Linn. (*Chaksu*)], dried fruits of *Ficus carica* Linn. (*Anjeer khushk*) and seeds of *Senna tora* (L.) Roxb. [Syn = *Cassia tora* Linn. (*Tukhm-e-panwar*)] as mentioned by Anonymous [13].

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 (Voucher Specimen Nos. S-143, S-144, F-189 and S-145). All these raw materials were observed carefully and foreign matters were removed. The raw materials were kept in oven at 40±2ºC for drying. The raw materials were powdered separately, passed through 355/180 # sieve and preserved in airtight glass containers. The raw materials were mixed in equal parts to get uniformly blended Sufoof [13].

Raw materials and formulation of SEB were standardized based on their pharmacognostic characters, physico-chemical properties, phytochemical analysis, heavy metal detection and HPTLC fingerprint profile.

Pharmacogenetic Study

Organoleptic evaluation refers to evaluation by color, odor, taste, texture etc. The organoleptic characters were carried out based on the method described by Siddique *et al*. [14]. Physicochemical contents such as the percentage of total ash, acid insoluble ash, water soluble ash, water soluble and alcohol soluble extractive values were performed as per standard methodology [15]. Fluorescence analysis was carried out as per standard procedure [16].

Preliminary phytochemical analysis

Preliminary phytochemical analysis of raw materials and formulation was performed as per standard methodology [17, 18].

Heavy metal analysis

Heavy metal analysis was carried out using Inductively Coupled Plasma - Atomic Emission Spectrometer (ICP-AES) instrument at Sophisticated Analytical Instrument Facility (SAIF) - IIT Powai, Mumbai, M.S., India for Lead, Arsenic, Cadmium and Mercury.

HPTLC fingerprint profile

A qualitative densitometric HPTLC analysis was performed for the development of characteristic fingerprint profile of all raw materials and formulation. The powders of raw material and formulation were soaked overnight separately in hydro alcoholic solvent and filtered. Filtrate was evaporated on water bath and reconstituted in ethanol. 5µl of extracts were spotted on pre-coated silica gel 60 F_{254} HPTLC plate using CAMAG Linomat V applicator. The HPTLC plates were then developed in glass twin trough chamber (20 cm x 10 cm) presaturated with mobile phase (Toluene: Methanol: GAA-7:3:0.02). The HPTLC plate was derivatized with Vanillin Sulphuric Acid. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and the images were captured under UV light at 254nm, 366nm and visible light.

Results and Discussion

Definition and characterization of the raw material is necessary prior to the assessment of the relevance of available efficacy and safety data. It is also important that the material used and available commercially should be consistent with the evaluated material[14]. Thus, there is an urgent need to evaluate such parameters which can be adopted by the pharmaceutical industries. In present work, an attempt has been made to standardize the raw materials and formulation of SEB using pharmacopeial and WHO guidelines.

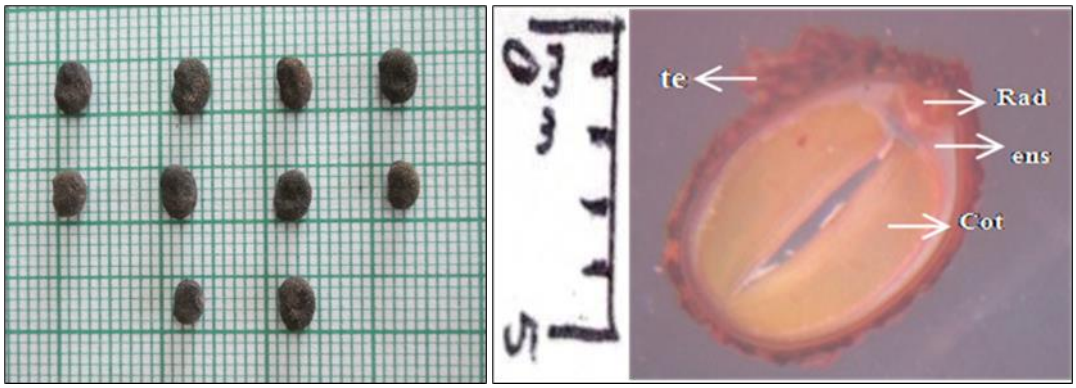
Macroscopy

Macroscopical features like shape, color, odor, taste, dimensions etc. of crude drugs are the primary pharmacognostic parameters for the correct identification. In the present study, seeds of *Cullen Corylifolium* (L.) Medik., seeds of *Chamaecrista absus* (L.) H. S. Irwin & Barneby., dried fruits of *Ficus carica* Linn. and seeds of *Senna tora* (L.) Roxb. Were described macroscopically in Table 1 and Fig. 1.

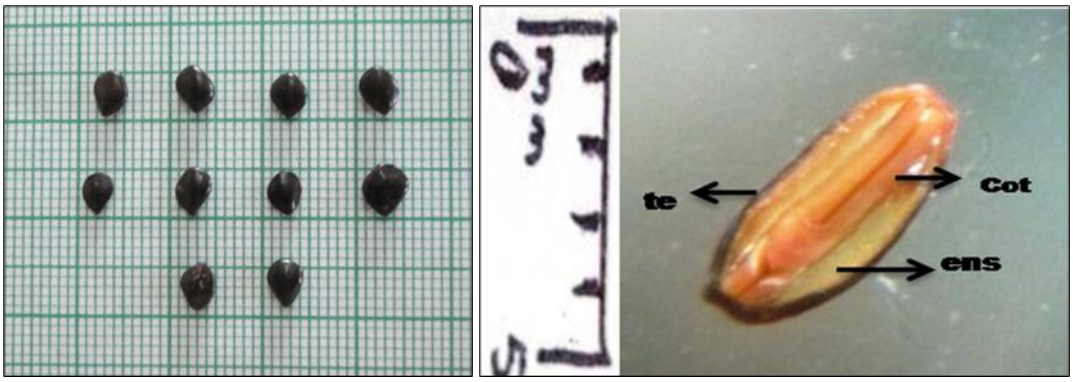
Table 1: Organoleptic and Macroscopical characterization of raw materials of SEB formulation

Raw Materials	Parameters					
	Color	Texture	Shape	Length (mm)	Width (mm)	Diameter (mm)
Pc	Brownish black	Glabrous	Oblong-flattened	3 ± 0.70	2.22 ± 0.44	NA
Ca	Black	Glossy	Oval	4 ± 0.30	3.2 ± 0.02	NA
Fc	Brown	Rough	Oval	NA	NA	25±0.02
Ct	Greenish brown	Glossy	Oblong	5 ± 0.92	3.01 ± 0.42	NA

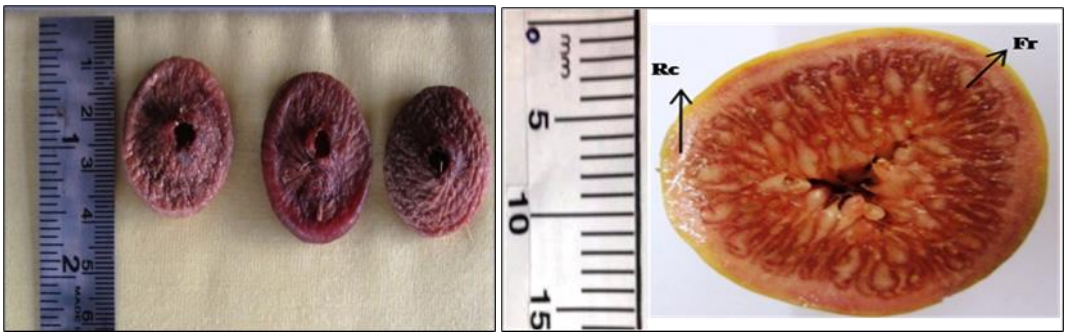
Key: *Cullen corylifolium* (L.) Medik. Seed, Ca- *Chamaecrista absus* (L.) H. S. Irwin & Barneby. Seed, Fc- *Ficus carica* Linn. Fruit, Ct- *Senna tora* (L.) Roxb. Seed, SEB – Sufoof-e-Bars formulation, NA- Not Applicable.



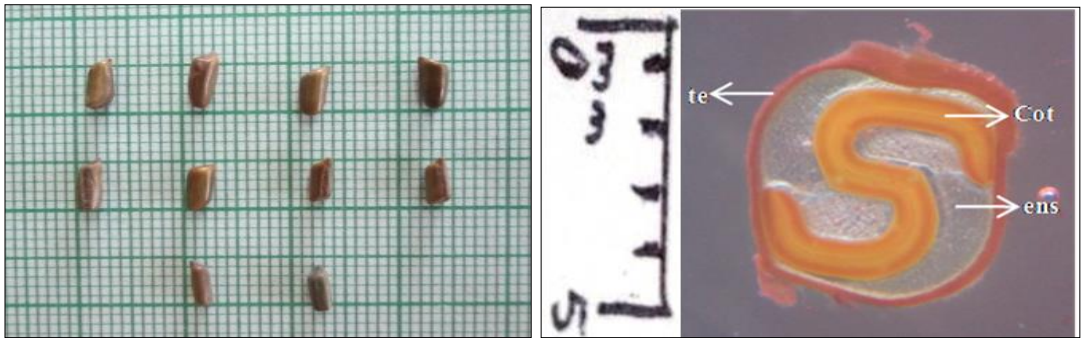
Cullen Corylifolium (L.) Medik. Seed



Chamaecrista absus (L.) H. S. Irwin & Barneby. seed



Ficus carica Linn. Fruit



Key: te-Testa, Cot-Cotyledon, ens-Endosperm, Rad-Radical, Fr-Florets, Rc-Rachis *Senna tora* (L.) Roxb. Seed

Fig 1: Macroscopy and Microscopy (Transverse Section) of raw materials of SEB formulations

Microscopy

Microscopy helps in exploring the internal structure of plant parts and is considered as a fascinating tool for correct taxonomy of the plants. The present microscopy study includes anatomical evaluation and powder microscopy of raw materials. Transversely cut sections of raw materials were observed under 10X of dissecting microscope to evaluate anatomical characters (Fig 1).

The transverse section of *Cullen Corylifolium* (L.) Medik. seed shows thick testa, radical, thin endosperm and cotyledons. The transverse section of *Chamaecrista absus* (L.) H. S. Irwin & Barneby. seed showed thick and horny

seed coat enclosing yellow coloured fleshy cotyledons, embedded in whitish endosperm (Figure 1). The transverse section of *Ficus carica* Linn. fruit showed presence of rachis and florets, the unisexual flowers (male, fertile female and sterile female) were arranged all over the inner surface of receptacle in cymose groups (Figure 1). The transverse section of *Senna tora* (L.) Roxb. seed showed presence of testa, endosperm and prominent 'S' shaped cotyledons (Figure 1).

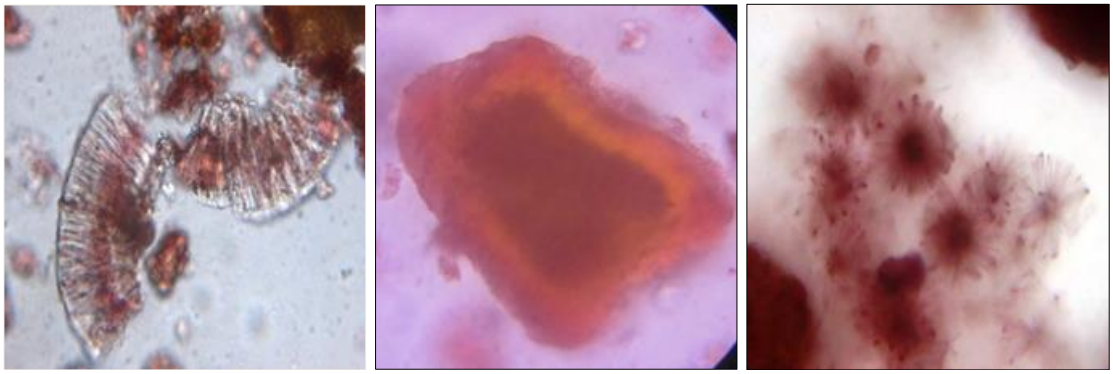
Powder Microscopy

The powdered raw materials were analyzed for anatomical characters. The powder microscopic analysis of raw materials revealed the presence of fragments of palisade cells, stone cell and rosette and cluster crystals of calcium oxalate in *Cullen Corylifolium* (L.) Medik. seed powder (Figure 2).

Chamaecrista absus (L.) H. S. Irwin & Barneby. seed powder showed presence of endospermic cells showing starch grains, fragment of cotyledon showing aleurone grains and transversely cut fragments of palisade layer of testa (Figure 2).

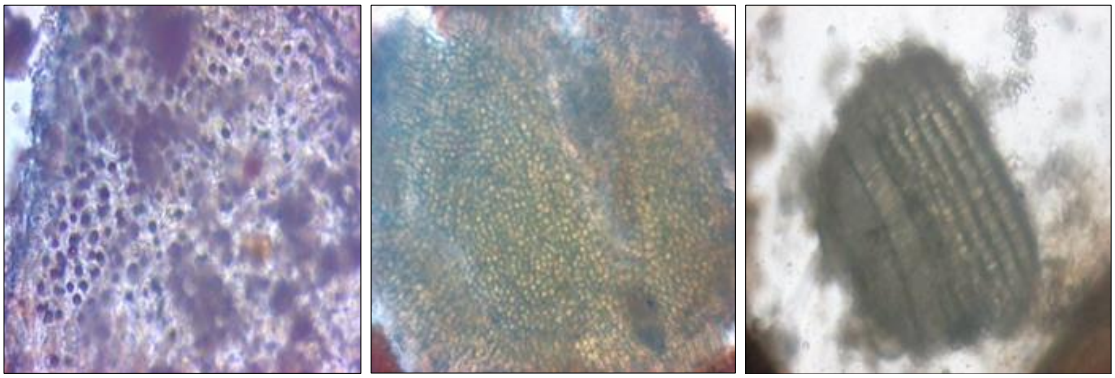
Ficus carica Linn. fruit powder showed presence of unisexual flowers (male, fertile female and sterile female), spiral vessels and trichome (Figure 2).

Senna tora (L.) Roxb. seed powder showed presence of endospermic cells embedded with aleurone grains, epidermal cells in surface view and transversely cut fragment of palisade layer of testa (Figure 2).



A **B** **C**

Cullen Corylifolium (L.) Medik. seed

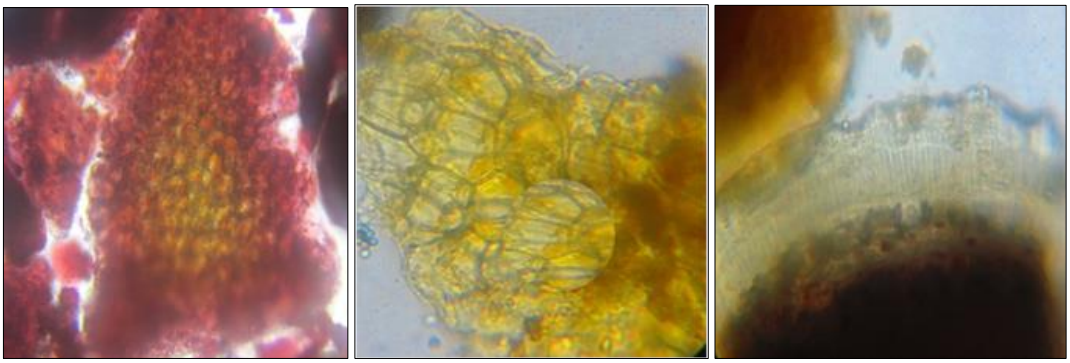


D **E** **F**

Chamaecrista absus (L.) H. S. Irwin & Barneby. Seed

G H I

Ficus carica Linn. Fruit



J K L

Senna tora (L.) Roxb. Seed

Fig 2: Powder Microscopy of raw materials of SEB formulation

Key: a-fragment of palisade cells, b- stone cell and c- Rosette and cluster crystals of calcium oxalate; d-Endospermic cells showing starch grains, e- fragment of cotyledon showing aleurone grains, f- Transversely cut fragments of palisade layer of testa; g-Unisexual flowers, h- Spiral vessels, i-Trichome; j- Endospermic cells embedded with aleurone grains, k- Epidermal cells in surface view, l- Transversely cut fragment of palisade layer of Testa

Organoleptic and Physicochemical Parameters

The organoleptic characters were observed for parameters like color, texture, Odour and taste. The results are shown in Table 2.

Table 2: Organoleptic analysis of raw materials and formulations of SEB

Raw Materials	Parameters			
	Color	**Texture**	**Odour**	**Taste**
Pc	Green	Moderately fine	Aromatic	Bitter
Ca	Light Brown	Moderately fine	Aromatic	Bitter
Fc	Brown	Moderately fine	Sweet	Sweet
Ct	Brownish Yellow	Fine	Pungent agreeable	Bitter
SEB	Yellowish brown	Moderately fine	Agreeable	slightly sweet and bitter

Key: Pc- *Cullen Corylifolium* (L.) Medik. Seed, Ca- *Chamaecrista absus* (L.) H. S. Irwin & Barneby. Seed, Fc- *Ficus carica* Linn. Fruit, Ct- *Senna tora* (L.) Roxb. Seed, SEB – Sufoof-e-Bars formulation

Physico-chemical constants *viz.* foreign matter, ash values (total ash, acid insoluble ash and water-soluble ash) and extractive values (water and alcohol soluble) were found in compliance with pharmacopeial limits (Table 3).

Table 3: Physico-chemical constant of raw materials and formulation of SEB

Parameters Raw Materials	Foreign Matter (%)	Total Ash (%)	Acid Insoluble Ash (%)	Water Soluble Ash (%)	Water Soluble Extractive (%)	Alcohol Soluble Extractive (%)
Pc	1.12 ± 0.47	4.650 ± 0.122	0.466 ± 0.057	2.56 ± 0.15	33.466 ± 0.768	29.520 ± 0.969
Ca	2.09 ± 1.31	3.250 ± 0.176	0.266 ± 0.057	1.70 ± 0.65	28.186 ± 4.001	6.329 ± 0.201
Fc	ND	4.016 ±0.075	0.866 ± 0.152	2.10 ± 0.17	68.106 ± 3.184	54.133 ± 3.873
Ct	0.61 ± 1.10	4.240 ± 0.069	0.3 ± 0.13	1.06 ± 0.30	27.066 ± 1.488	8.346 ± 0.482
SEB Formulation	NA	3.933 ± 0.273	0.366 ± 0.115	1.53 ± 0.28	41.36 ± 0.486	22.053 ± 0.394

Key: Pc- *Cullen Corylifolium* (L.) Medik. seed, Ca- *Chamaecrista absus* (L.) H. S. Irwin & Barneby. seed, Fc- *Ficus carica* Linn. fruit, Ct- *Senna tora* (L.) Roxb. seed, SEB – Sufoof-e-Bars formulation, NA- Not Applicable, ND- Not Detected

Preliminary phytochemical analysis of raw materials and formulation of SEB revealed the presence of various primary and secondary phytoconstituents (Table 4).

Table 4: Preliminary Phytochemical Analysis of raw materials and SEB formulation

Phytoconstituents	Pc			Ca			Fc			Ct				SEB Formulation	
	AE	EE	PE	AE	EE	PE	AE	EE	PE	AE	EE	PE	AE	EE	PE
Acid compounds	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aleurone grains	+	+	-	+	+	+	+	-	-	+	+	-	+	-	-
Amino acid	-	-	-	+	+	+	-	+	-	-	+	-	+	+	-
Carbohydrates	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+
Fats and fix oils	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+
Proteins	+	+	+	-	+	-	-	-	-	+	+	-	-	-	-
Starch	-	-	-	+	+	-	+	+	-	-	+	-	+	+	-
Alkaloids	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-
Anthraquinone	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-
Essential oil	-	-	-	+	+	-	-	+	-	-	+	-	-	+	-
Flavonoids	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-
Glycosides	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Mucilage	+	+	-	+	+	-	+	+	-	+	-	-	+	+	-
Resins	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
Saponins	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+
Tannins	+	+	-	-	+	-	-	+	-	+	+	-	+	+	+
Triterpenoids	+	+	+	-	-	-	-	+	-	-	-	-	+	+	+

Key: Pc- *Cullen Corylifolium* (L.) Medik. seed, Ca- *Chamaecrista absus* (L.) H. S. Irwin & Barneby. seed, Fc- *Ficus carica* Linn. fruit, Ct- *Senna tora* (L.) Roxb. seed, SEB – Sufoof-e-Bars formulation, AE – Aqueous Extract, EE – Ethanolic Extract, PE – Petroleum ether Extract, + – Present and - – Not detected.

Fluorescence Analysis

The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material. The powder drug exhibit different fluorescence characters due to the presence of different functional groups. The color observed under UV 366 nm, 254 nm and visible light are tabulated in Table 5.

Table 5: Fluorescence Analysis of raw materials and SEB formulation

TESTS FOR FLUROSCENCE	*Pc*			*Ca*			*Fc*			*Ct*			SEB		
	ODL	UV. 254	UV. 366	ODL	UV. 254	UV. 366	ODL	UV. 254	UV. 366	ODL	UV. 254	UV. 366	ODL	UV. 254	UV. 366
Powder as such	G B	B	G	Bf	Gr	F Gr	Br	Bf Br	Bf	Bf Br	Gr	Y Br	G B	Gr G	G Bf
Powder + Nitrocellulose	G B	Gr	G	Bf	Gr	F Gr	Br	Bf Br	Bf	Bf Br	Gr	Y Br	G B	Br	Br
Powder + 1N NaOH in Methanol	O Br	B	R Br	B Y	Gr B	L Y	D Br	B	B	B Br	Br	B	O Br	Br	Br
Powder + 1N NaOH in methanol + nitrocellulose in amyl acetate	YG	D G	G	Bf	Gr	B	L Br	Bf	W Bf	Y Br	D Pr	Bf	P YG	B	B
Powder + 1N HCl	D Br	B	D G	Y B	B	Bf G	D Br	Bf Gr	Du G	D Br	B	B	D Br	B	B
Powder + 1N HCl + Nitrocellulose in amyl acetate	Y Br	B	D Bu	O Br	Br B	B	Br	B	Bf G	Br	B	B	Y Br	Gr	Gr
Powder + 1N NaOH in water	B Br	D Bu	G	Bf Br	D Bu	Gr	B	B	B	B	B	B	B Br	Gr	Wh
Powder + H_2SO_4 (1:1)	Y	Gr G	Gr G	Bf Br	Gr	Gr	Bf Br	Gr	W	Bf Br	Gr	Bf	Y	B	G Bf
Powder + 1% Picric acid	B Br	D Bu	B	B	Bu	Gr	B Br	B	B	B Br	B	B	B Br	B	B
Powder + Acetic acid	Y Br	G	L G	Bf	Bf	B	Br	G	Gr	Br	Gr G	G Bf	Y Br	Gr	F Gr
Powder + 5% Iodine	Y Br	G	L G	Y Gr	Bf	L G	Br	Bf	Bf	Br	Br	Br	Y Br	Gr	F Gr
Powder + 5% $FeCl_3$	Br	G	Gr	O	Br B	GY	Br	Br	Gr	O Br	Br	Br	Br	GrB	L Y
Powder + 25% NH_3 + HNO_3	Br	B	B	O Br	B	B	O Br	B	B	R Br	B	B	Br	Y G	Y
Powder + Methanol	O Br	B	G	B Br	B	Bf	Br	B	Gr	D Br	B	B	O Br	B	G
Powder + Conc. HNO_3	GB	B	G	Bf	Gr	F Gr	Br	Bf Br	Bf	Bf Br	Gr	Y Br	G B	Gr	G
Powder + 10% Potassium dichromate solution	GB	Gr	G	Bf	Gr	F Gr	Br	Bf Br	Bf	Bf Br	Gr	Y Br	G B	Gr	B
Powder + 50% KOH	O Br	B	R Br	B Y	Gr B	L Y	D Br	B	B	B Br	Br	B	O Br	D Bu	B

Key: D- Dark, L-Light, P- Pale, Bf- Buff, Du-Dull, F- Fluorescent, B- Black, Br- Brown, Y- Yellow, O-Orange, G-Green, Gr- Grey, R-Red, GB- Greenish black, Orange brown, YG- yellow green, Bu- Blue, W- White, Pr- Purple ODL- Ordinary day light Pc-

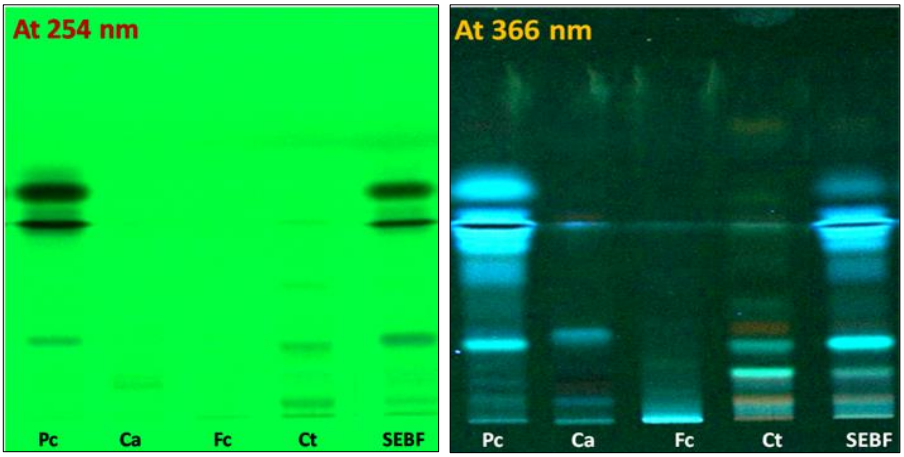
Cullen Corylifolium (L.) Medik. seed, Ca- *Chamaecrista absus* (L.) H. S. Irwin & Barneby. seed, Fc- *Ficus carica* Linn. fruit, Ct- *Senna tora* (L.) Roxb. seed, SEB – Sufoof-e-Bars formulation

Heavy metal Analysis

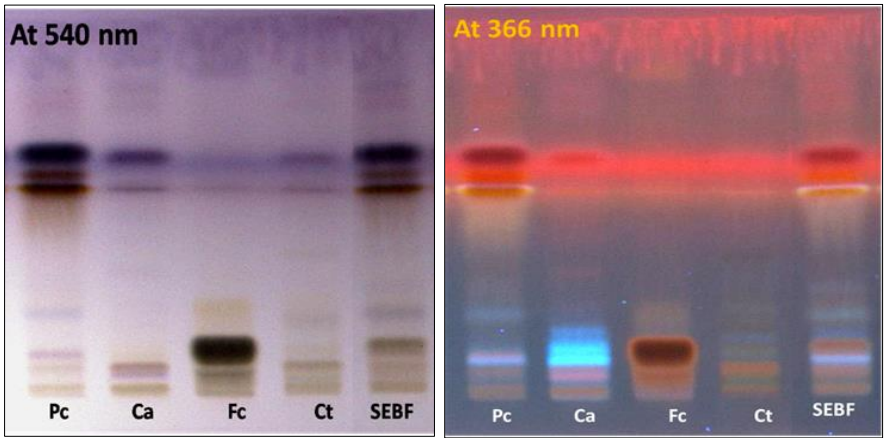
Heavy metals *viz*. Lead, Arsenic, Cadmium and Mercury were not detected in all the raw materials used as well as in SEB formulation.

HPTLC fingerprint profile

HPTLC fingerprint profile is a valuable quality assessment tool for the evaluation of botanical materials, it allows the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods as the spots are well resolved and it is only chromatographic method offering the option of presenting the results as an image. The HPTLC unique fingerprint profile (Chromatogram) was developed using different solvent systems. Solvent system containing Toluene: Methanol: GAA (7:3:0.02) gave well resolved bands. The developed chromatogram was observed before derivatisation under 254 and 366 nm whereas same was observed after derivatisation under 366 and 540 nm (Figure 3).



Before Derivatisation



Key: Pc-Psoralea corylifolia, Ca- Cassia absus, Fc-Ficus carica, Ct- Cassia tora, SEBF- Sufoof-e-Bars Formulation

After Derivatisation

Fig 3: HPTLC fingerprint profile of raw materials and formulation of SEB

Conclusion

The present study on raw materials and formulation of Sufoof-e-Bars provides important information regarding the quality control parameters which are very essential to assure safety and efficacy of this formulation. The values obtained for the physico-chemical parameters for the formulation can also be adopted to lay down pharmacopeial standards to be followed in traditional preparation of Sufoof-e-Bars to assure batch to batch consistency. A routine use of such scientific technique will lead to standardization of the Unani medicines to some extent and would help in building confidence in use of this formulation for clinical treatment of vitiligo.

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