Development of Quality Standards of a Single Unani Drug - Habb-e-Balsan

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Abstract

abb-e-Balsan consists of dried fruits of *Commiphora opobalsamum* (L.) Engl. belongs to family Burseraceae. Unani medicines are prepared using different parts of the plant materials such as seeds, fruits, flowers, stems, bark, wood, leaves, roots and gums etc., The dried fruits of the drug in Unani System of Medicine are used as expectorant and emmenagogue and also to cure diseases of the urinary tracts and neurological disorders. The present study deals with pharmacognostical (to identify), physico-chemical (purity) and WHO parameters (safety) of the samples of Habb-e-Balsan procured from Chennai and Hyderabad. Pharmacognostical studies show the presence of epidermal cells with occasional anomocytic stomata, mesocarpic parenchyma cells, stone cells up to 100µ, druses of calcium oxalate crystals up to 35µ and cotyledonary parenchyma cells. Physico-chemical data obtained included moisture content (8.68% & 7.95%), total ash (8.52% & 8.31%), acid in-soluble ash (1.37% & 1.41%) and solubility in alcohol (10.08% & 9.93%) and water (20.01% & 20.43%). TLC studies of chloroform and alcohol extracts showed identical spots at 254nm, 366nm and in visible light (Vanillin Sulphuric acid reagent). WHO parameters such as microbial content (TBC, TFC, Enterobacteriaceae, Salmonellae and *Staphylococcus aureus*) and the heavy metals (As, Cd, Pb and Hg) were found within the permissible limit. The aflatoxins B_1, B_2, G_1 and G_2 were not detected from the drug samples Habb-e-Balsan.

Key Words: Habb-e-Balsan, Microscopy, Powder microscopy, Physico-chemical, TLC and WHO parameters

Introduction

Pharmacopoeial study of a single drug is the systematic study which involves official title or vernacular names, biological sources and family, geographical sources, collection, processing, macroscopical characters, microscopical characters, chemical test and quality control parameters (Kokate *et al*., 2000).

Habb-e-Balsan consists of dried fruits of *Commiphora opobalsamum* (L.) Engl. (Syn. *Balsamodendron opobalsamum* Kunth., *B. gileadensis* Kunth., & *C. gileadensis* (L.) Engl.,), (Family : Burseraceae). The dried fruit is also called as *carpobalsamum*. The fruit is reddish grey, and in the size of a small pea, with an agreeable and aromatic taste; the seeds of the fruits are solitary, yellow and grooved down one side (Hooper, 1937). The dried fruit of the plant contains

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various organic constituents such as linoleic, oleic, stearic and palmitic acids, sitosterol, stigmasterol, cholesterol, campesterol and α-spinasterol (Rastogi and Mehrotra, 1993).

In Unani medicine the fruits of the drug is used as Mudir-e-Haiz (Emmenagogue), Munaffis-e-Balgham (Expectorant) (Khare, 2007), Mujaffif (Desiccant / Siccative), Mulattif (Demulcent), Kasir-e-Riyah (Carminative), Moharrik (Stimulant), Muqawwi-e-Meda wa Ama (Stomachic and Intestinal tonic), Zimad (Liniment) and also in neurological affections (Ahmed *et al*., 2005; Nadkarni, 1976). The drug Habb-e-Balsan is used in the preparation of compound formulations viz. Jawarish-e-Kamooni Kabir, Jawarish-e-Kafoor and Habb-e-Barmak (Anonymous, 2006 & 2007). The present study was aimed to evaluate the pharmacopoeial standards of the fruit of Habb-e-Balsan using standard protocol.

Materials and Methods

a. Pharmacognostical study

Macroscopic study: The dried fruits were collected from the local raw drug dealers of Chennai and Hyderabad. The fruits were identified using the available literature of Flora of Tropical East Africa (Gillett, 1991).

Microscopic study: The free hand sections of the fruit (T. S) were taken, stained with safranin and mounted in glycerine. Hand diagrams were made using Camera Lucida (Khandelwal, 1998).

Powder microscopy: The coarse powder of the dried fruit was treated with various chemical reagents like phloroglucinol + HCl and Jeffrey's reagent (Johansen, 1940) for clearing the tissues to study the various elements.

b. Chemical analysis

Physico-chemical studies like total ash, acid insoluble ash, alcohol and water solubility and loss on drying at 105ºC were carried out as per the standard methods (Anonymous, 1987).

c. Thin layer chromatography

Preparation of extract: Powders of the fruits (2g) were extracted with 20ml of chloroform and alcohol solvents separately. The extracts were concentrated and made up to 5ml in volumetric flask and used for thin layer chromatographic studies.

Thin Layer Chromatography profile: TLC profile of chloroform and ethanol extracts were performed using the solvent systems of toluene: ethyl acetate, 9 : 1 and 1 : 1.3 respectively on pre-coated silica gel 60 F_{254} TLC plate (E. Merck) as adsorbent. After drying, the plates were examined under UV – 254nm and 366nm and observed the spots. Then plates were dipped in vanillin-sulphuric acid reagent and heated at 105ºC till appeared the bright spots (Wagner *et al*., 1984).

d. Quality control parameters

The quality control parameters viz. microbial load by serial dilution, heavy metals by Atomic Absorption Spectrophotometer and aflatoxins by High Pressure Liquid Chromatography were carried out using the standard methods of WHO (Anonymous, 1998) & AOAC guidelines (Anonymous, 2000).

Results and Discussions

a. Pharmacognostical Study

Macroscopic: Fruits reddish brown dehiscent drupe (Fig. 1), ovate somewhat compressed, 10mm long and 7mm wide with a pointed smooth nut marked on one side by a longitudinal furrow; the fleshy pericarp splitting into 2 values disclosing a 2 locular 1-2 seeds stone usually surrounded at the base by a brightly coloured fleshy pseudoaril; pericarp composed of fused epicarp and mesocarp (Fig. 2); cotyledons flat or plicate, entire as broad as long; odour agreeable and aromatic taste.

Microscopic: T. S. of fruit shows (Fig.3) an epicarp with epidermis single layered, consisting of small thick walled polygonal parenchyma cells covered with a thin layer of cuticle; mesocarp consisting of three different regions, outer region consisting of 3 to 4 layers of rectangularly elongated polygonal parenchyma cells, middle region consisting of big cells of oval to rectangular polygonal parenchyma cells followed by inner region consisting of few layers of smaller parenchyma cell; a few resinous canals, vascular bundles and numerous druses of calcium oxalate crystals found scattered in the mesocarpic region; endocarp (Fig. 4) consisting of two regions, outer region consisting of 2 to 4 layers of thick walled sclereids or stone cells followed by inner region consisting of 5 to 7 layers of thick walled sclereids or stone cells separated by a single layer of thin walled parenchyma cells.

T. S. of the seed shows (Fig. 5) testa and cotyledons; testa consisting of outer layer of thick walled epidermal cells with druses and inner layer of small thin

walled parenchyma cells in between the two 3 to 4 layers of parenchyma cells with vascular tissues; endosperm present with a single layer of polygonal parenchyma cells filled with starch grains; cotyledons plicate, epidermis of the cotyledons consisting of single layer of polygonal parenchyma cells on both the surfaces; 3 to 4 layers of polygonal parenchyma cells followed by a single layer of palisade parenchyma cells on the lower side of cotyledons.

Powder microscopy: Epidermis of the fruit in surface view with occasional anomocytic stomata; mesocarpic parenchyma cells in surface view; vessels with spiral thickening upto 20µ; druses of calcium oxalate crystals upto 35µ; sclereids of stone cells upto 70m; stone cells with wavy walls in surface view; outer layer of testa in surface view with druses; inner layer of small thin walled parenchyma cells; outer layer of cotyledons (epidermis) in surface view and cotyledonary parenchyma cells (Fig. 6).

b. Chemical analysis

Analytical data of both the samples shows 8.68% & 7.95% of moisture content. Total Ash contents of the samples were 8.52% & 8.31% and 1.37% & 1.41% of acid in-soluble ash shows the siliceous matter in the drug. Alcohol soluble extractive (10.08% & 9.93%) represents the extraction of polar constituents like phenols, tannins, glycosides, alkaloids and flavonoids. The water soluble extractive (20.01% & 20.43%) denotes the presence of inorganic contents. The results of physico-chemical parameters are shown in Table (1).

c. Thin Layer Chromatography

The R_f values of the samples of chloroform and alcohol extracts are shown in Table-2 and 3. The plates were visualized using vanillin-sulphuric acid reagent and heated at 105º till appeared the colored spots (Fig. 7 and 8).

d. Quality control parameters

The microbial content and heavy metals in the drug samples were found within the permissible limit (Table–4 and 5). The aflatoxins were not detected from the drug samples (Table–6).

Habb-e-Balsan

Commiphora opobalsamum (L.) Engl.

Fruit

Fig. 1 - Fruit
Surface View

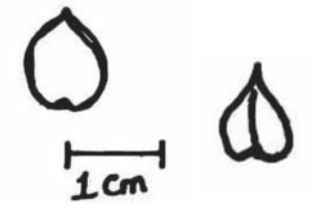


Fig. 2 - T.S of fruit
A Diagrammatic sketch

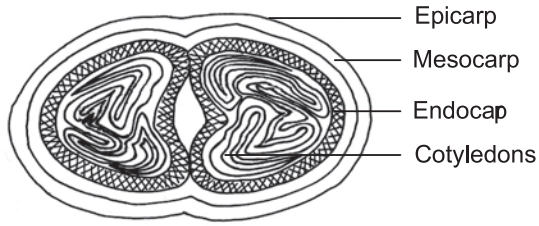


Fig. 3 - T. S. of Fruit (Epicarp & Mesocarp)

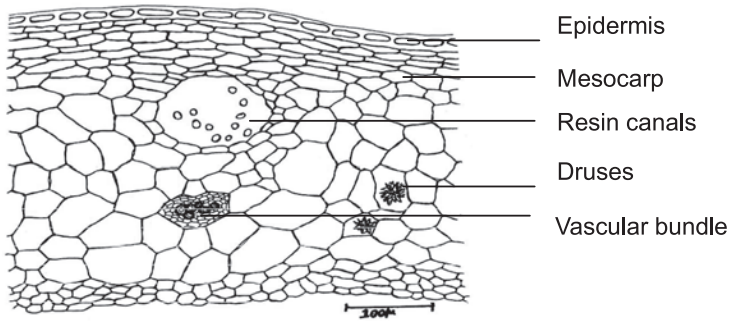


Fig. 4 - T. S. of Fruit (Endocarp)

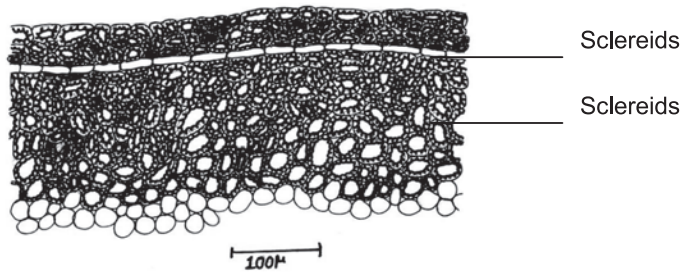
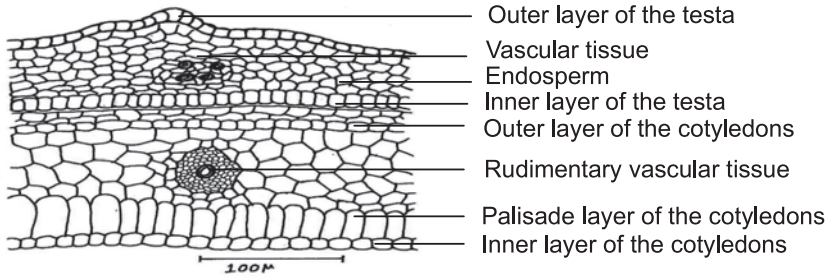


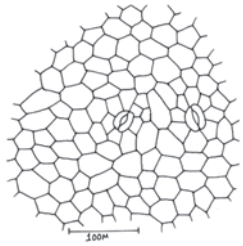
Fig. 5 - T. S. of Seed (Testa & Cotyledons)



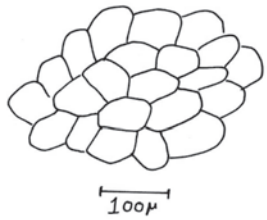
Habb-e-Balsan
Commiphora opobalsamum (L.) Engl.
Fruit

Fig. 6 – Powder Microscopy

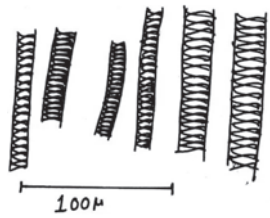
Epidermal cells of the fruit in surface view with occasional anomocytic stomata



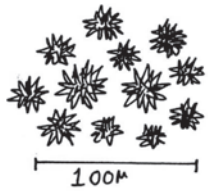
Mesocarpic parenchyma cells in surface view



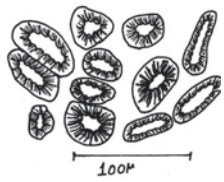
Vessels with spiral thickening



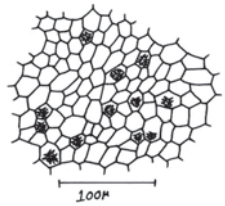
Druses of calcium oxalate crystals



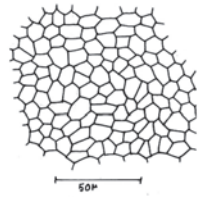
Sclereids



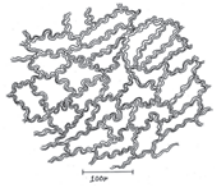
Outer layer of the testa in surface view with druses



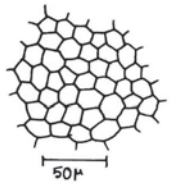
Inner layer of the testa in surface view



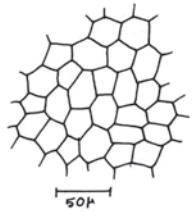
Stone cells with wavy walls in surface view



Epidermis of the outer layer of cotyledonary parenchyma cells

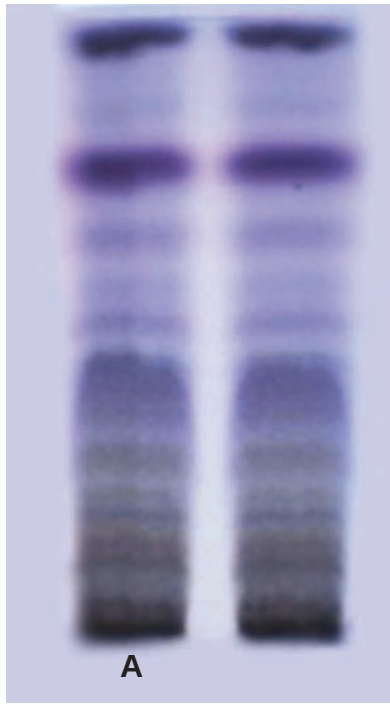


Cotyledonary parenchyma cells



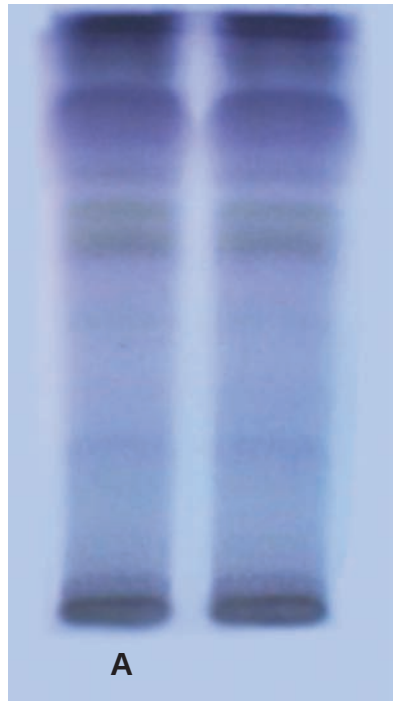
Thin Layer Chromatography

Fig. 7



Chloroform extract
Solvent system: Toluene : Ethyl acetate
(9 : 1)
Detector: V. S. Reagent

Fig. 8



Alcohol extract
Solvent system: Toluene : Ethyl acetate
(1 : 1.3)
Detector: V. S. Reagent

A = Chennai & B = Hyderabad

Table-1 : Physico-chemical parameters

S. No.	Parameters	Results (n = 3) + S.D.	
		Chennai	Hyderabad
1.	Foreign matter (%)	1.29	1.09
2.	Loss on drying at 105ºC (%)	8.68	7.95
3.	Ash (%)	8.52	8.31
4.	Acid insoluble ash (%)	1.37	1.41
5.	Alcohol soluble extractives (%)	10.08	9.93
6.	Water soluble extractives (%)	20.01	20.43

Table-2 : TLC data of the chloroform extract

Solvent system	R_f Values		
Toluene : Ethyl acetate (9 : 1)	UV 254 nm	UV 366 nm	V. S. Reagent
	0.57 Light pink	0.52 Greenish blue	0.84 Grey
	0.48 Light pink	0.32 Light blue	0.75 Pink
	0.37 Light pink	0.23 Light blue	0.65 Violet
	0.26 Pink		0.56 Violet
	0.19 Pink		0.48 Violet
	0.15 Pink		0.41 Blue
			0.29 Brown
			0.19 Blue
			0.12 Brown

Table–3 : TLC data of the alcohol extract

Solvent system	R_f Values		
Toluene : Ethyl acetate (1 : 1.3)	UV 254nm	UV 366nm	V. S. Reagent
	0.83 Pale pink	0.91 Greenish Blue	0.91 Grey
	0.76 Pale pink	0.73 Light blue	0.84 Blue
	0.71 Pale pink	0.56 Light blue	0.71 Violet
	0.65 Pale pink		0.66 Yellowish green
	0.61 Pale pink		0.60 Brown
	0.46 Pale pink		0.48 Blue
			0.26 Blue

Table–4 : Microbial load

S. No.	Parameter Analyzed	Results		WHO Limits
		Chennai	Hyderabad	
1	Total Bacterial Count	2300 CFU/gm	1900 CFU / gm	105 CFU/gm
2	Total Fungal Count	100 CFU/gm	150 CFU / gm	103 CFU/gm
3	Enterobacteriaceae	Absent	Absent	103 CFU/gm
4	*Salmonella* Spp.	Absent	Absent	Nil
5	*Staphylococcus aureus*	Absent	Absent	Nil

Table–5 : Heavy metals

S. No.	Parameter Analyzed	Results		WHO & FDA Limits
		Chennai	Hyderabad	
1	Arsenic	Nil	Nil	10 ppm
2	Cadmium	Nil	Nil	0.3 ppm
3	Lead	0.026ppm	0.019ppm	10 ppm
4	Mercury	Nil	Nil	1.0 ppm

Table–6 : Estimation of Aflatoxins

S. No.	Aflatoxins	Results		Detection Limit
		Chennai	Hyderabad	
1	B_1	Not detected	Not detected	DL 1.0 ppb
2	B_2	Not detected	Not detected	DL 0.5 ppb
3	G_1	Not detected	Not detected	DL 1.0 ppb
4	G_2	Not detected	Not detected	DL 0.5 ppb

Conclusion

The pharmacognostical studies shows the presence of epidermal cells with occasional anomocytic stomata, mesocarpic parenchyma cells, stone cells upto 100µ, druses of calcium oxalate crystals upto 35µ and cotyledonary parenchyma cells. The safety parameters were found to be within the permissible limit. The evaluated pharmacopoeial study will help to lay down the scientific standards of the drug for inclusion in Unani Pharmacopoeia.

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