Pharmacognostical and Physico-chemical Studies of Ood-e-Balsan (*Commiphora gileadensis* (L.) C. Chr.) – A Unani Drug

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Abstract

Present study was undertaken to standardize the Unani single drug Ood-e-Balsan (*Commiphora gileadensis* (L.) C. Chr. Fam. – Burseraceae). Ood-e-Balsan (wood) is used in Unani System of Medicine to cure the ailments of epilepsy and nervine disorders. The drug samples were procured from raw drug dealers of Chennai and Hyderabad and standardized by employing pharmacognostical and physico-chemical methods. The quality control parameters were carried out as per WHO and AOAC guidelines to assess the quality of drug samples. Macroscopical studies revealed the presence of layers of papery bark on the outer surface of the wood. Microscopical studies showed the presence of cork cells, secondary cortex, secretory canals, stone cells, xylem vessels, uniseriate and multi-seriate rays and prisms of calcium oxalate crystals. The physico-chemical data showed that the drug contains moisture (8.53%, 8.68%), total ash (1.86%, 1.90%), acid insoluble ash (0.42%, 0.39%) and solubility in alcohol (2.66%, 2.74%) and water (4.23%, 4.12%). TLC studies of chloroform and alcohol extract showed various spots at 254nm, 366nm and visible light (Vannilin – Sulphuric acid reagent). The quality control results revealed the absence of microbial load, heavy metal and aflatoxins from the drug. The obtained data of pharmacognostical and physico-chemical parameters will provide the referential supporting information in the identification and standardisation of the crude drug Ood-e-Balsan.

Keywords: Ood-e-Balsan, Pharmacognosy, Physico-chemical, WHO parameters

Introduction

Ood-e-Balsan consists of wood of *Commiphora gileadensis* (L.) C. Chr. (Syn:- *Commiphora opobalsamum* (L.) Engl., *Balsamodendron opobalsamum* Kunth.) (Fam. – Burseraceae); is a small evergreen non-thorny woody tree native to Southern Arabia; the plant is known under several names: Persia – Ud-i-Balsan; English – Balsam tree, Balsam of Mecca, Balsam of Gilead. The dried branchlets or wood known as xylobalsamum, is pinkish and heavy in texture (David Hooper, 1937). The different parts of the drug viz. resin, fruit, bark and wood/twig have many therapeutic properties and acts as astringent, demulcent, expectorant, stomachic, carminative and stimulant (David Hooper, 1937). The ancient usage viz. sore throat, cough, laryngitis, chronic bronchitis and inflammations due to rheumatism and arthritis of this plant have been reported by Arabians (Chopra *et al.,* 1956). The literature survey reveals that

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the plant contains various chemical constituents viz., Friedelin, canophyllal, oleanolic acid, mearnsetin, quercetin, syringic acid (Abbas *et. al.,* 2007), cycloartan-24-ene-1α,2α,3α-triol, 3β-acetoxycycloartan-24-ene-1α,2α-diol, 1α-acetoxycycloartan-24-ene-2α,3β-diol, 3β-isovaleroyloxycycloartan-24-ene-1α,2α-diol, cycloartan-24-ene-1α,3β-diol, cycloartan-23*E*-ene-1α,2α,3β,25-tetrol, 24*R*,25-epoxycycloartane-1α,2α,3β-triol, 24*S*,25-epoxycycloartane-1α,2α,3β-triol and cycloartan-24-ene-1α,2α,3β-triol (Shen *et al.,* 2008, Jules Janick, 2012).

In Unani system of medicine, the drug is used in the treatment of ailments like epilepsy (Sara), nervine disorders (Zof-e-Asab) (Khare, 2007) and used as one of the raw drug in the preparation of formulations like Jawarish-e-Jalinoos, Jawarish-e-Falafili, Sabadaritoos and Zuroor-e-Gaozaban (Anonymous, 2006).

The therapeutic efficacy of the plant is also proved by reported pharmacological activities like hepatoprotective (Al-Howiriny, 2004), anti-inflammatory, analgesic, antipyretic and diuretic activity (Tawfeq *et al.,* 2004).

The present study was an attempt to standardize the drug samples by employing pharmacognostical, physico-chemical and quality control parameters to ascertain the quality of Ood-e-Balsan.

Materials and Methods

Collection of the plant material

Raw drug samples were procured from raw drug dealers of Chennai and Hyderabad. The drug samples were obtained in cut pieces covered with layers of papery bark.

Pharmacognostical studies

The collected samples of the drug were identified and botanical identification of the drug samples were carried out using available literature. Free hand sections of the drug were taken, stained in safranine and mount in glycerine. The coarsely powdered drug samples were used for powder microscopical studies and the powders were treated with various chemical reagents like phloroglucinol + HCl and jeffrey's reagent (Johansen D.A., 1940) for clearing the tissues to study the vascular elements. The microphotography in different magnifications of all necessary cells and tissues were taken using MIPS attached with trinocular microscope.

Physico-chemical parameters

Physico-chemical parameters like foreign matter, total ash, acid in-soluble ash, loss on drying at 105°C, solubility in alcohol and water were carried out as per standard method (Anonymous, 1987).

TLC analysis

Preparation of extract

Powdered drug samples (2g) were extracted separately with chloroform and alcohol using Soxhlet apparatus. The extracts were filtered through Whatman No. 1 filter paper, concentrated and made up to 5ml in standard flask separately.

TLC developing method

Chloroform and alcohol extracts were applied on pre-coated silica gel 60 F_{254} TLC plate (E Merck) as absorbent and developed the plates using the solvent systems Toluene : Ethyl acetate (9 : 1) and (1 : 1) respectively (Wagner, 1984).

Analysis of quality control parameters

The WHO parameters like microbial load, heavy metals and aflatoxins were carried out using standard methods of WHO (Anonymous, 1998) & AOAC guidelines (Anonymous, 2000).

Table I Physico-chemical parameters

Parameters	Chennai	Mean value	Hyderabad	Mean value
Foreign matter	Nil	--	Nil	--
Moisture (% W/W)	7.81 8.86 8.91	8.53	8.15 8.88 9.02	8.68
Alcohol soluble matter (% W/W)	2.60 2.64 2.75	2.66	2.66 2.74 2.82	2.74
Water soluble matter (% W/W)	4.08 4.24 4.36	4.23	3.94 4.18 4.25	4.12
Total ash (% W/W)	1.78 1.85 1.95	1.86	1.80 1.91 1.99	1.90
Acid in-soluble ash (%W/W)	0.38 0.40 0.48	0.42	0.35 0.38 0.44	0.39

Table II Rf Values of Chloroform extract

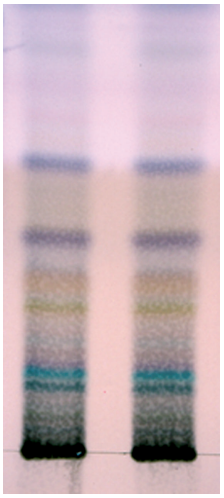
Solvent system & Detector	Rf Values		
 Toluene : Ethyl acetate (9 : 1) V. S. reagent	UV 254nm	UV 366nm	V. S. Reagent
	0.87 Light pink	0.87 Light blue	0.87 Light grey
	0.68 Pink	0.63 Blue	0.64 Grey
	0.59 Light pink	0.55 Light blue	0.47 Violet
	0.48 Light pink	0.43 Blue	0.39 Blue
	0.36 Pink	0.33 Fluorescent blue	0.31 Yellowish green
	0.29 Pink	0.25 Light blue	0.17 Blue
	0.21 Light pink	0.16 Fluorescent blue	0.13 Grey
	0.16 Pink		
	0.13 Light pink		
	A – Chennai & B – Hyderabad		

Table III Rf Values of Alcohol extract

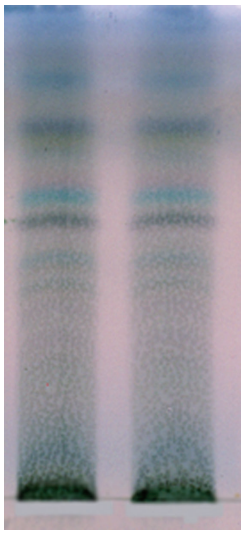
Solvent system & Detector	Rf Values		
 Toluene : Ethyl acetate (1 : 1) V. S. reagent	UV 254nm	UV 366nm	V. S. Reagent
	0.87 Light pink	0.85 Blue	0.95 Grey
	0.71 Pink	0.72 Blue	0.83 Blue
	0.63 Light pink	0.64 Blue	0.76 Violet
	0.59 Pink	0.58 Fluorescent blue	0.72 Yellow
	0.56 Light pink	0.51 Blue	0.63 Pink
	0.48 Light pink	0.44 Blue	0.61 Blue
	0.35 Light pink	0.35 Blue	0.56 Grey
	0.14 Light pink	0.14 Light blue	0.48 Blue
			0.43 Blue
			0.26 Grey
	A – Chennai & B – Hyderabad		

Table IV Microbial Load

S. No.	Parameter Analyzed	Results		WHO Limits
		Chennai	Hyderabad	
1	Total Bacterial Count	2,600 CFU/gm	2,300 CFU/gm	105 CFU/gm
2	Total Fungal Count	Nil	Nil	103 CFU/gm
3	*Enterobacteriaceae*	Absent	Absent	103 CFU/gm
4	*Salmonella* Spp.	Absent	Absent	Nil
5	*Staphylococcus aureus*	Absent	Absent	Nil

Table V Heavy Metals

S. No.	Parameter Analyzed	Results		WHO & API Limits
		Chennai	Hyderabad	
1	Arsenic	Nil	Nil	3 ppm
2	Cadmium	Nil	Nil	0.3 ppm
3	Lead	0.029 ppm	0.037 ppm	10 ppm
4	Mercury	Nil	Nil	1 ppm

Table VI Estimation of Aflatoxins

S. No.	Aflatoxins	Results		Detection Limit
		Chennai	Hyderabad	
1	B1	Not detected	Not detected	(DL: 1.0 ppb)
2	B2	Not detected	Not detected	(DL 0.5 ppb)
3	G1	Not detected	Not detected	(DL 1.0 ppb)
4	G2	Not detected	Not detected	(DL 0.5 ppb)

Ood-E-Balsan – *Commiphora gileadensis* (L.) C. Chr.

Wood

Fig. 1: Wood - Surface View

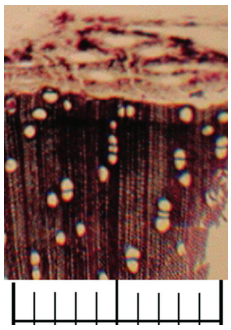


Fig. 2: Wood – T. S.

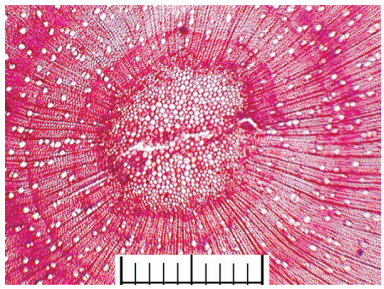


Fig. 3: T. S. of Wood – Central pith region

T. S. of Wood

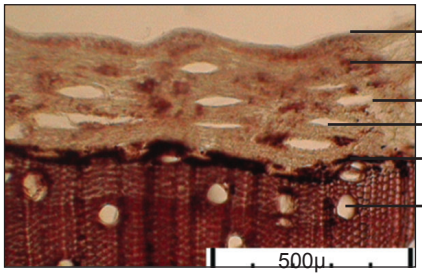


Fig. 4

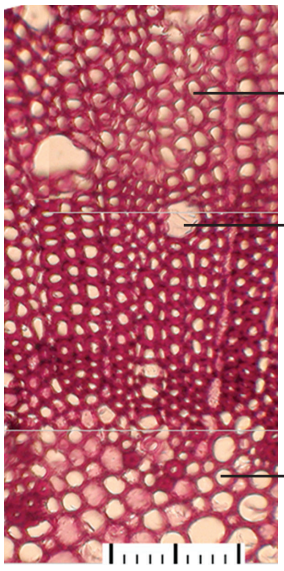


Fig. 5

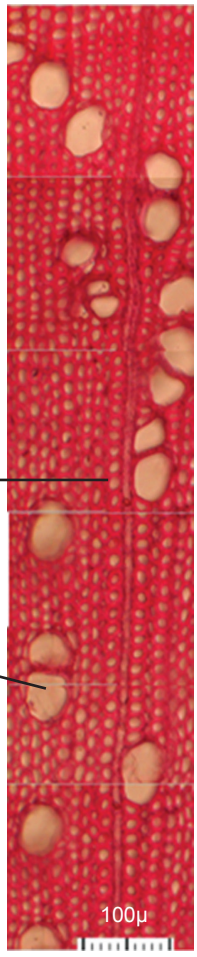


Fig. 6

Ood-E-Balsan – *Commiphora gileadensis* (L.) C. Chr.

Wood

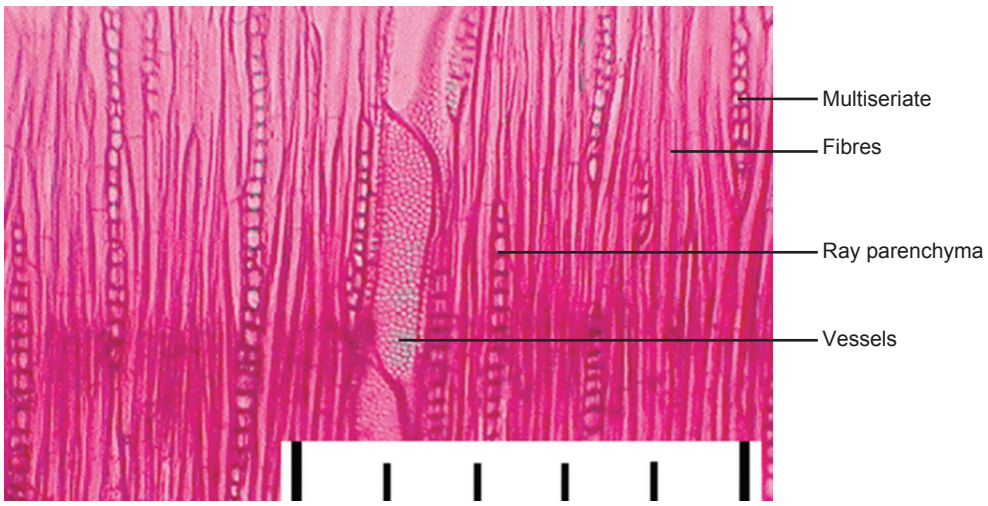


Fig. 7: T. L. S. of Wood

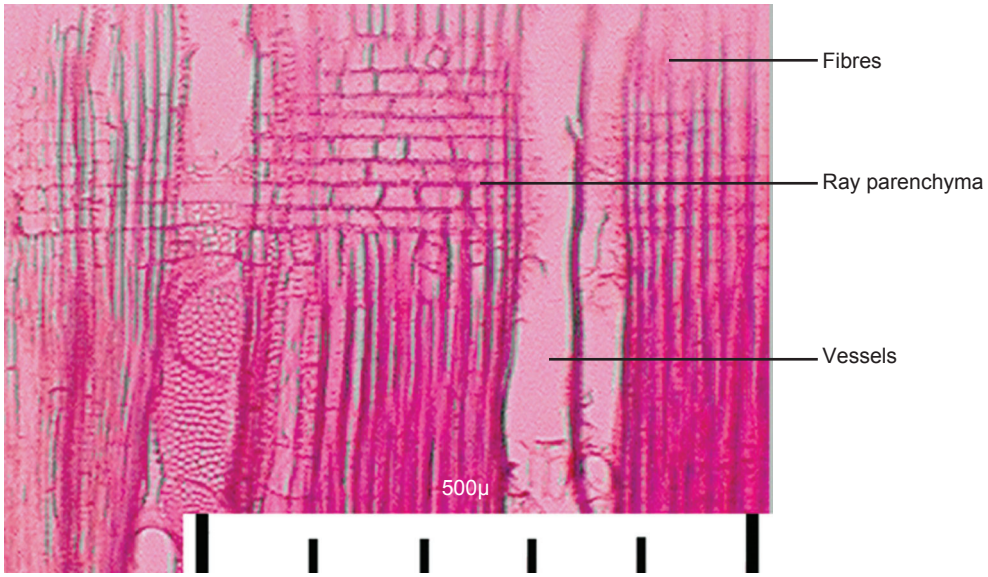
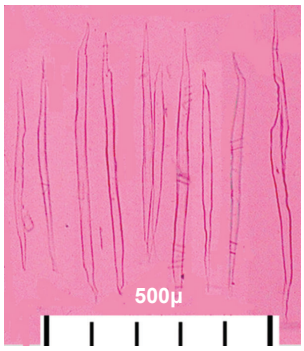


Fig. 8: R. L. S. of Wood

Ood-E-Balsan – *Commiphora gileadensis* (L.) C. Chr.

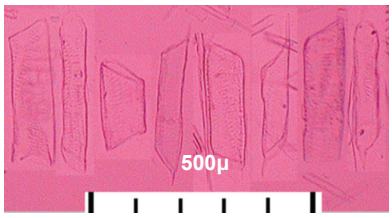
Wood



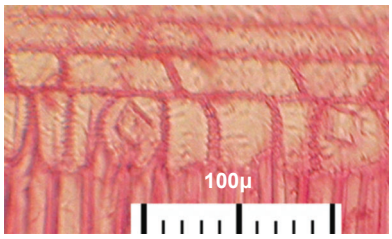
Fibres

Fibres

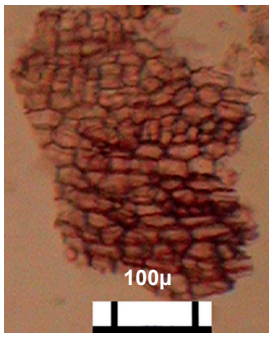
Tracheids



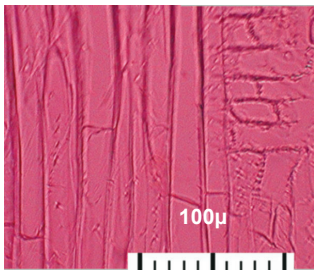
Pitted vessels



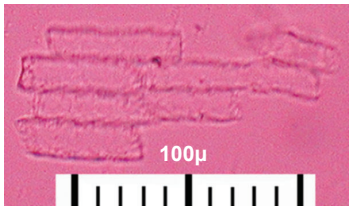
Prismatic crystals of calcium oxalate



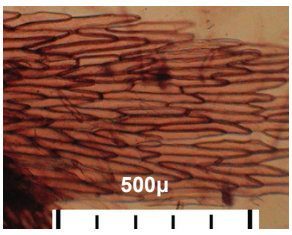
Cork cells



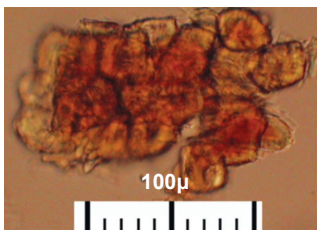
Septate fibres



Medullary ray parenchyma cells



Thin walled cortical cells in surface view



Stone cells

Fig. 9: Powder

Results and Discussion

Macroscopic: The cut pieces of wood of about 5 to 10cm in thickness, the wood is pinkish and heavy in texture, the cut pieces covered with layers of papery bark (Fig. 1); aromatic smell and tasteless.

Microscopic: The Transverse section (T.S.) of wood shows circular in outline (Fig. 2 & 3); secondary growth present; epidermis consisting of single layer of thick walled rectangular cells; cork consisting of few layers of rectangular tabular cells; secondary cortex consisting of few layers of thin walled tangentially elongated parenchyma cells traversed longitudinal by secretary canals (Fig. 4), very few stone cells present in the this region; vascular cylinder closed; phloem towards outside and xylem towards inside; xylem vessels arranged in radial groups (Fig. 5); each xylem vessels moderately small to medium sized; vessels solitary or in multiples of 2 or 3, occasionally 4 or 5 cells; xylem parenchyma paratracheal varying from scanty to vasicentric and occasionally with some diffuse; parenchyma as sheath around the vessel; rays uniseriate but occasionally 2 to 4 cells wide, some of the enlarged cells containing prismatic crystals of calcium oxalate, central small pith consisting of parenchyma cells (Fig. 6).

The Tangential longitudinal section (TLS) (Fig. 7) of wood shows elongated pitted vessels, fibres, medullary rays mostly uniseriate and rarely multiseriate. The Radial longitudinal section (RLS) (Fig. 8) of wood shows broad lumen elongated pitted vessels, xylem parenchyma, fibres, medullary rays arranged perpendicular to other elements.

Powder: Light brown; pitted vessels of length upto 500µ and breadth upto 100µ with oblique end walls and occasionally tailed (Fig. 9); fibres lignified thick walled of length upto 750µ and breadth upto 30µ; fibre tracheids with pitted thickening of length upto 600µ and breadth upto 40µ; septate fibres; stone cells present; thin walled elongated cortical parenchyma cells with secretary canals in surface view, medullary ray parenchyma cells; cork cells in surface view; prismatic crystals of calcium oxalate upto 25µ in ray parenchyma cells.

Physico-chemical parameters

Moisture content of the drug samples was 8.53% and 8.68%. Alcohol soluble extractive (2.66% and 2.74%) indicates the presence of polar phyto-constituents and water soluble extractive (4.23% and 4.12%) indicates the presence of inorganic constituents. The obtained physico-chemical data of the drug samples are shown (Table –I).

Thin Layer Chromatography analysis

Thin Layer Chromatographic studies of chloroform and alcohol extract of both the samples showed identical spots in various detectors which indicate the similarity of the drug. The R_f values of both the extracts are shown (Table – II & III).

Quality control parameters

Studies of quality control parameters were performed using WHO and AOAC methods. The microbial load and heavy metals were found within the permissible limit (Table – IV & V). The aflatoxins were not detected from the drug samples (Table – VI).

Conclusion

The pharmacognostical and physico-chemical data have shown that the collected drug samples from Chennai and Hyderabad are identical and can be used for the preparation of Unani formulations. The results of quality control parameters revealed that the drug samples taken for study are free from toxic substances like microbes, heavy metals and aflatoxins.

Acknowledgement

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