Standardization of Habb-Ul-Aas (*Myrtus communis* Linn., Fruits): A Unani drug**

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Abstract

abb-ul-Aas botanically known as *Myrtus communis* Linn., belongs to family Myrtaceae. The fruit is one of the important single drugs used in Unani System of Medicine. Fruit contains the wide range of phyto-constituents and therapeutically used in the ailments of diarrhea, dysentery, internal ulceration, rheumatism, bronchitis, cough, palpitation and headache. Present study was aimed to authenticate the fruit of *Myrtus communis* and to evaluate its scientific standards by employing pharmacognostical, physico-chemical and quality control methods. Microscopic studies show the presence of schizolysigenous oil glands in the surface view of epidermal cells, mesocarpic parenchyma cells, stone cells, druses of calcium oxalate crystals and cotyledonary parenchyma cells. The fruit contains moisture (14.62%), total ash (3.54%), acid in-soluble ash (0.25%) and solubility in alcohol (21.65%) and water (25.56%). TLC studies of chloroform and alcohol extracts show various spots at 254nm, 366nm and visible light (Vanillin – Sulphuric acid reagent). The quality control parameters such as microbial load, heavy metal, aflatoxins and pesticidial residues were not detected from the drug.

Key words: Habb-ul-Aas, *Myrtus communis* L., Pharmacognostical, Physico-chemical, Quality control methods.

Introduction

Herbal medicines are prepared using a variety of plant materials like leaves, stem, roots, barks, fruits and seeds. Plant material contains many biological active ingredients which are responsible for treating mild or chronic ailments. As the plant materials have many therapeutic values, they have to be investigated using modern sophisticated analytical instruments and also by employing the scientific parameters. To ascertain the quality of a drug three attributes viz. authenticity, purity and assay are desirable.

Habb-ul-Aas is an important herbal drug used in Unani system of medicine to cure the variety of ailments like gastric ulcer, diarrhea, dysentery, vomiting, deep sinus, leucorrhoea and also for cosmetic purpose (like hair fall control) (Sabiha *et al*., 2011). The drug is used in the preparation of various Unani

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formulations viz. Jawarish-e-Jalinoos, Majoon-e-Khabs-ul-Hadeed, Majoon-e-Muqawwi-e-Rahem, Majoon-e-Sangdana Murgh, Majoon-e-Albula, Majoon-e-Bawaseer and Maaski (Anonymous, 2006, 2007, 2011).

Fruit contains many phyto-chemicals like citric acid, malic acid, resin, tannin, fixed oil, phenols, flavonoids, anthocyanins, arabinosides, kaempferol, quercetin, myricetin, caffeic acid, myricetin 3-O-rhamnoside, esculetin-6-O-glucoside, hesperetin-7-O-rhamnoglucoside, hesperetin-2-O-methylchalcone-4-O-rhamnoglucoside (Montoro *et al.,* 2006; Hinou *et al.,* 1988; Martin *et al*., 1999). The reported various pharmacological activities like anti-oxidant (Serce *et al*., 2010), anti-diabetic, anti-mutagenic and anti-microbial activity have been proved the therapeutic efficacy of the drug (Sabiha *et al*., 2011).

Present study was an attempt to standardize the drug by employing pharmacognostical, physico-chemical and quality control parameters to ascertain the quality of fruit of Habb-ul-Aas.

Materials and Methods

(i) Collection of the plant material

Raw drug samples were procured from different raw drug dealers of Chennai, Hyderabad and New Delhi. The fruits (Chennai –DSM70A- ; Hyderabad – DSM70B-; New Delhi – DSM70C) were authenticated by the botanist and deposited in the Museum of Drug Standardization Research Unit, Regional Research Institute of Unani Medicine, Chennai , Tamil Nadu, India.

(ii) Pharmacognostical studies

Botanical identification of the fruit was carried out using available literature (Brandis D, 1988; Kritikar and Basu, 1998). The pharmacognostical studies viz. macroscopic, microscopic and powder microscopy were carried out using standard method (Johansen, 1940). Free hand sections of the fruit were taken and microscopical drawings were made using Camera Lucida and observations were recorded.

(iii) 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

(i) Preparation of extract

Powdered drug samples (2g) soaked in chloroform and alcohol separately for 24 hours and filtered. The filtrates were concentrated and made upto 5 ml in standard flask separately.

(ii) Method of developing the plates

Chloroform and alcohol extracts were applied on precoated 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 chloroform : methanol (19 : 1) respectively (Wagner, 1984).

(iii) Quality control parameters

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

Results and Discussion

Macroscopic: Fruits berry, small, black, ellipsoidal or globose upto 13mm length and 9mm width with 4 to 5 partite persistent calyx at the top; surface wrinkled; seeds 1 to many seeded each seed ivory or pale yellow to white, very hard, looks like reniform, length upto 4mm and breadth upto 3mm (Fig. 1), taste sweet and no characteristic odour.

Microscopic

Calyx: T. S. of persistent calyx (Fig. 2) shows a single layer of epidermal cells on both the surfaces, cortex consisting of several layers of polygonal parenchyma cells, schizolysigenous oil glands and druses of calcium oxalate crystals present, vascular tissue present in the centre.

Fruit: T. S. of fruit (Fig. 3) circular in outline; 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 zones (Fig. 4), outer zone consisting of 2 to 4 layers of rectangular, thick walled, polygonal parenchyma cells; middle zone consisting of big cells of oval to polygonal, thin walled, parenchyma cells with intercellular spaces; most of the mesocarpic

cells filled with reddish brown contents; a vascular bundles found scattered in the mesocarpic regions; inner zone consisting of few layers of thin walled, small, parenchyma cells compare to the other region; numerous druses of calcium oxalate crystals scattered in this region; schizolysigenous oil glands present in the epicarp and mesocarp region; endocarp (Fig. 5) consisting of 10 to 15 layers of thick walled stone cells; cotyledons consisting of compactly arranged polygonal parenchyma cells with a single layer of epidermis on both the surfaces, cotyledonary parenchyma cells filled with aleurone grains and oil globules.

Powder: Pale brown; epidermal cells in surface view (Fig. 6), mesocarpic parenchyma cells in surface view (Fig. 7), stone cells of length upto 150µ and breadth upto 70µ (Fig. 8); druses of calcium oxalate crystals upto 30µ (Fig. 9); cotyledonary parenchyma cells in surface view (Fig. 10) and spiral vessels upto 10µ (Fig. 11).

Physico-chemical parameters

Moisture content of the drug shows 14.62% and alcohol soluble extractives (21.65%) might be due to the extraction of polar constituents. Water soluble extractives (25.63%) indicate the presence of inorganic constituents. Physico-chemical data of the drug are shown (Table –1).

Thin Layer Chromatography analysis

Thin Layer Chromatographic studies of chloroform and alcohol extracts of all the three region samples showed identical spots in various detectors. The R_f values of both the extracts were tabulated in Table 2 & 3.

Quality control parameters

The microbial contents were found to be within the permissible limit (Table – 4). The other parameters such as heavy metals, aflatoxin and pesticide residue were not detected from the drug (Table – 5, 6 & 7) which indicates the drug is free from toxic substances.

HABB-UL-AAS (*Myrtus communis* Linn.)

Fruit

Fig. 1 - Surface view

Entire Fruit Seed

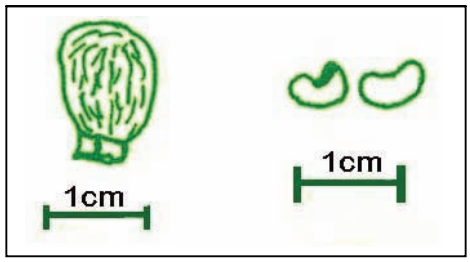
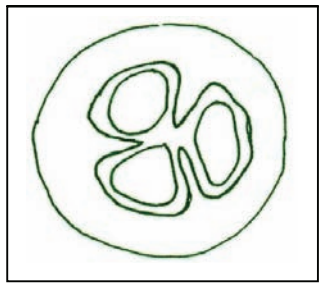


Fig. 3 - T.S. of Fruit
A Diagramatic Sketch



T. S. of Fruit

Fig. 4 - Epicarp and Mesocarp

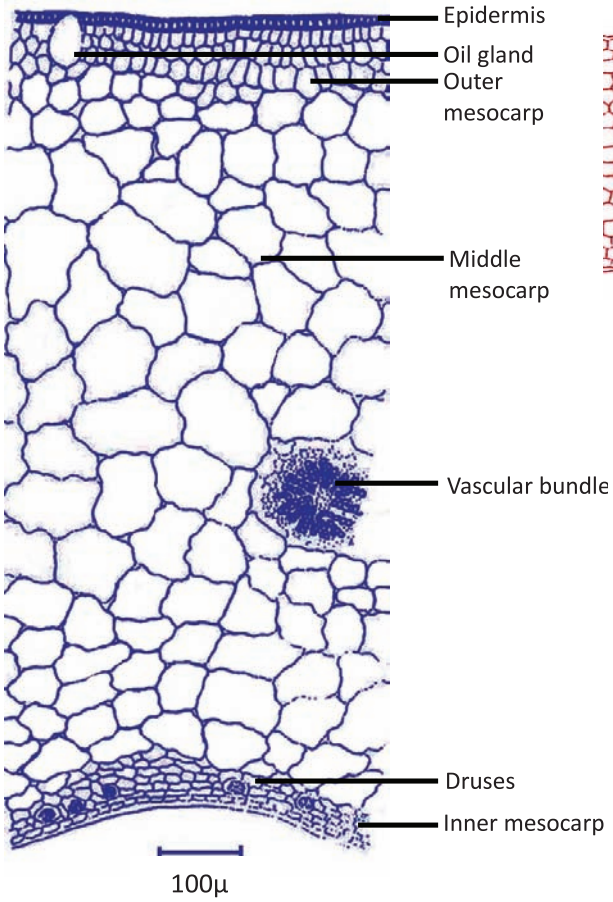


Fig. 2 - T. S. OF CALYX

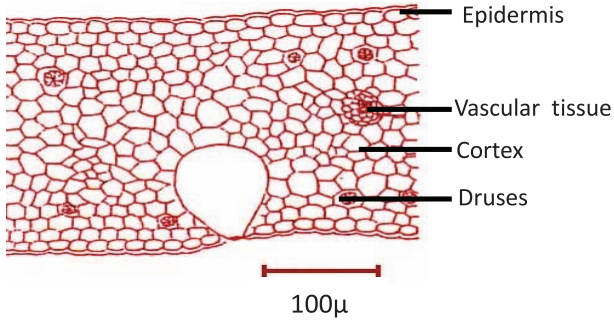
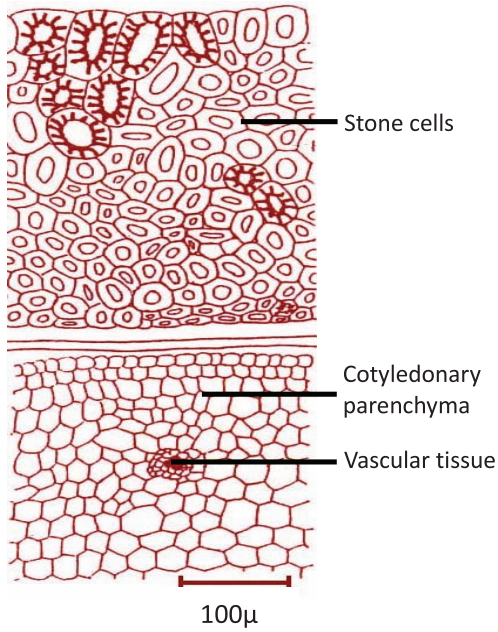


Fig. 5 - T. S. OF SEED



HABB-UL-AAS (*Myrtus communis* Linn.)

Fruit

Powder

Fig. 6 - Epidermal cells in surface view

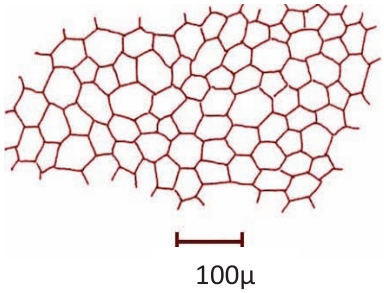


Fig. 7 - Mesocarpic parenchyma cells in surface view

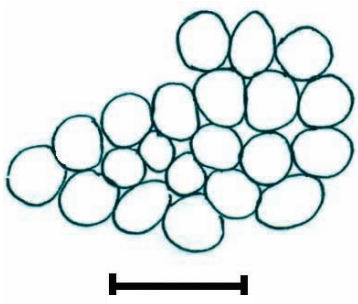


Fig. 8 - Druses of calcium oxalate crystals

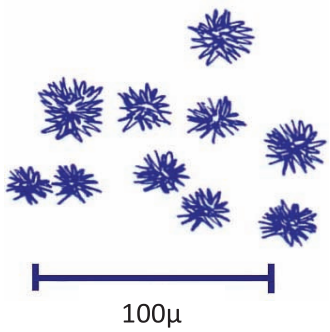


Fig. 9 - Stone cells

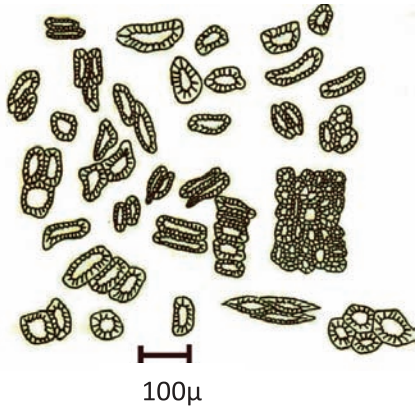


Fig. 10 - Cotyledonary parenchyma cells in surface view

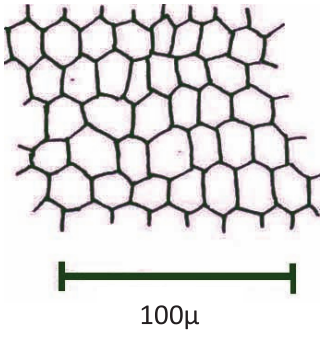


Fig. 11 - Spiral vessels

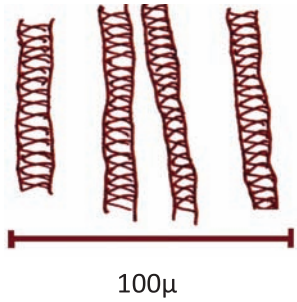


Table 1: Physico-chemical parameters

Parameters	Source of samples					
	Chennai	Mean value	Hyderabad	Mean value	Delhi	Mean value
Foreign matter (% W/W)	Nil	--	Nil	--	Nil	--
Alcohol soluble matter (% W/W)	21.44 21.52 21.60	21.52	21.20 21.68 21.76	21.55	21.83 21.88 21.96	21.89
Water soluble matter (% W/W)	25.40 25.48 25.68	25.52	25.24 25.36 25.40	25.33	25.76 25.84 25.88	25.83
Total ash (% W/W)	3.29 3.53 3.84	3.55	3.45 3.58 3.63	3.55	3.42 3.54 3.59	3.52
Acid in-soluble ash (%W/W)	0.25 0.26 0.27	0.26	0.23 0.27 0.29	0.26	0.21 0.23 0.26	0.23
Moisture (% W/W)	14.64 14.71 14.75	14.70	14.77 14.84 14.89	14.83	14.28 14.31 14.37	14.32

Table 2: R_f Values of chloroform extract

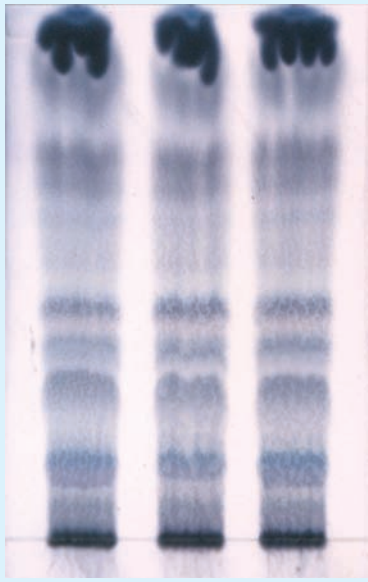
Solvent system & Detector	Rf Values		
	UV 254nm	UV 366nm	Visible light (V. S. Reagent)
	0.93 Pink	0.83 Light blue	0.83 Light grey
	0.82 Pink	0.63 Red	0.71 Violet
	0.72 Light pink	0.49 Blue	0.65 Grey
	0.67 Light pink	0.38 Red	0.60 Light grey
	0.53 Yellowish green	0.19 Violet	0.50 Light grey
	0.42 Violet		0.42 Violet
	0.36 Yellowish green		0.35 Grey
	0.28 Pink		0.28 Violet
	0.19 Pink		0.15 Violet
Toluene: Ethyl acetate (9 : 1) V. S. reagent	A – Chennai; B – Hyderabad; C - Delhi		

Table 3: R_f Values of alcohol extract

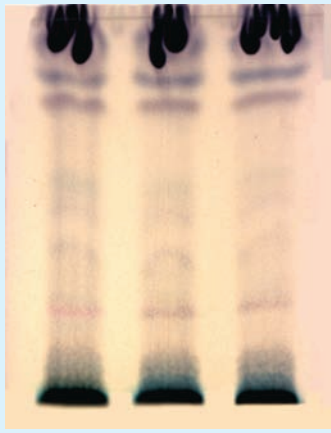
Solvent system & Detector	Rf Values		
 Chloroform : Methanol (19 : 1) V. S. reagent	UV 254nm	UV 366nm	Visible light (V. S. Reagent)
	0.84 Light pink	0.84 Light blue	0.84 Violet
	0.56 Light pink	0.31 Blue	0.76 Grey
	0.23 Pink	0.17 Light blue	0.45 Light grey
	0.17 Yellowish green		0.35 Grey
	0.12 Yellowish green		0.23 Violet
			0.12 Grey
	A – Chennai; B – Hyderabad; C - Delhi		

Table 4: Microbial load

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

Table 5: Heavy metals

S. No.	Parameter Analyzed	Results	WHO & FDA Limits
1	Arsenic	Nil	10 ppm
2	Cadmium	Nil	0.3 ppm
3	Lead	0.0142 ppm	10 ppm
4	Mercury	Nil	1.0 ppm

Table 6: Estimation of Aflatoxins

S. No.	Aflatoxins	Results
1	B1	Not detected
2	B2	Not detected
3	G1	Not detected
4	G2	Not detected

Table 7: Analysis of Pesticide Residues

S. No.	Pesticide Residues	Results
1	Organo Chlorine Group	ND
2	Organo Phosphorus Group	ND
3	Acephate	ND
4	Chlordane	ND
5	Dimethoate	ND
6	Endosulphan	ND
7	Endosulfan	ND
8	Endosulfon	ND
9	Ethion	ND
10	Endosufon sulphate	ND
11	Fenthion	ND
12	Lindane	ND
13	Methoxychlor	ND
14	Phorate sulfoxide	ND
15	Phorate sulfone	ND
	ND – Not detected	

Conclusion

The pharmacognostical parameters which are reported for the first time will be useful in setting some diagnostic indices for the identification of the fruit of Habb-ul-Aas. Results of the comparative study on physicochemical, TLC and quality control parameters of three region samples will help to lay down the pharmacopoeial standards.

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