

## DRUG STANDARDISATION

### Standardisation of Homoeopathic drug - *Syzygium Jambos* (L.) Alston.

P. Padma Rao<sup>1</sup>, P. Subramanian<sup>1</sup>, P.R. Reddy<sup>1</sup> and H.C. Gupta<sup>2</sup>

<sup>1</sup>Drug Standardisation Unit, Hyderabad

<sup>2</sup>Central Council for Research in Homoeopathy, New Delhi

*Syzygium jambos*, a small tree belonging to the family Myrtaceae is a potential drug in Homoeopathy. The drug is useful in clinical conditions like acne, pimples, nausea, comedones, headache, heartburn, abdominal colic, diarrhoea and fever.

The seeds are up to 2.5 cm long, ellipsoid to oblong, narrowed at middle. Seed coat is rough, thick, sclerotic and papery, made of tangentially elongated lignified cells with dense contents and separating from the endosperm. Cortex is 20 – 40 layered, interspersed with groups of sclerenchyma fibers. The endosperm possess large secretory cavities in the hypodermal areas. The endosperm is filled with starch grains often encapsulated in the plastids. A few tracheary bundles are present in the endosperm.

Powder microscopical and organoleptic characters are presented. Physico-chemical parameters of raw drugs viz., extractive values, ash values, formulation, besides wt. per mL, total solids, alcohol content and TLC and UV studies are given for the mother tincture.

**Keywords:** *Syzygium jambos*; seeds; pharmacognosy; physico-chemical studies; chromatography (TLC)

### Introduction

*Syzygium jambos* (syn. *Eugenia jambos* L.), popularly known as 'Rose apple', is a small tree belonging to the family Myrtaceae. It is cultivated in many parts of India.<sup>1</sup> The seeds of the tree are used for preparation of homoeopathic medicine. The drug was introduced and proved by C. Hering and mentioned in Homoeopathic Materia medica.<sup>2,3,4</sup> In Homoeopathy, the drug is useful in clinical conditions like acne, pimples, nausea, comedones, headache, heartburn, abdominal colic, diarrhoea and fever.<sup>2,3</sup>

Chemically, the plant is reported to possess several phenolic compounds viz., Quercetin and Myricetin 3-O-beta-D-xylopyranosyl (1-->2) alpha-L-rhamnopyranosides.<sup>5</sup> Ellagic acid derivatives viz., 3,3',4'-tri-O-methyl ellagic acid-4-O-beta-D-glucopyranoside and 4-O-acetyl-3,3',4'-tri-O-methyl ellagic acid has been extracted from the leaves of *Eugenia jambos*.<sup>6</sup> Phloretin 4'-O—methyl ether,

myriganone G and myriganone B have shown anti oxidant activity<sup>7</sup> are also present in the plant extract.

A perusal of literature reveals no pharmacognostic and physico-chemical standards have been reported for the drug. In view of the importance of the drug in homoeopathy, pharmacognostic and physico-chemical studies of seeds are carried out for laying standards.

### Materials and Methods

#### Pharmacognostic studies

The seeds of *Syzygium jambos*, were obtained from Survey of Medicinal Plants and Collection Unit, Nilgiris, Tamil Nadu.[Voucher specimen No.8364 collected at private garden, Coimbatore dt.10.6.2009]. The seeds were boiled at 80°C for 10 minutes, cooled and fixed in F.A.A. (Formalin-Acetic-Alcohol). Mature seeds were dehydrated through TBS series and embedded in paraffin wax. Sections, cut between 8-10 microns, were stained with crystal violet–basic fuchsin combination<sup>8</sup>. The microscopic characters of powder were observed by boiling the powdered drug in distilled water, stained in saffranin and mounted in glycerine.

<sup>1</sup> Corresponding author:

Dr. P. Padma Rao,

Drug Standardisation Unit,

O.U.B. 32, Road No.4, Hyderabad -7.

e-mail ID: padmarao.pogaku@yahoo.com

### Physico-chemical studies

The air dried seeds were coarsely powdered to 10/44 sieve size and the powder was subjected to determination of moisture content (loss on drying at 105°C), total ash, acid insoluble ash, water soluble ash and extractability in water and alcohol following official methods. Mother tincture was prepared by percolation method as per Homoeopathic Pharmacopoeia of India.<sup>9</sup>

Mother tincture was analyzed for its physico-chemical, chromatographic and spectroscopic absorbance. All the chemicals and solvents of analytical grade (AR) were used. Silica gel 60 F254 pre-coated plate (Merck, Germany) was used for Thin Layer Chromatography (TLC) and work was carried out at room temperature.<sup>10-11</sup> The TLC plate was developed in chloroform-methanol (17:3, v/v) as solvent system and was visualized in UV short and long wave length and also in day light. The mother tincture was diluted with methanol for Ultraviolet-visible spectroscopy. The maximum absorption is recorded.

### Observations

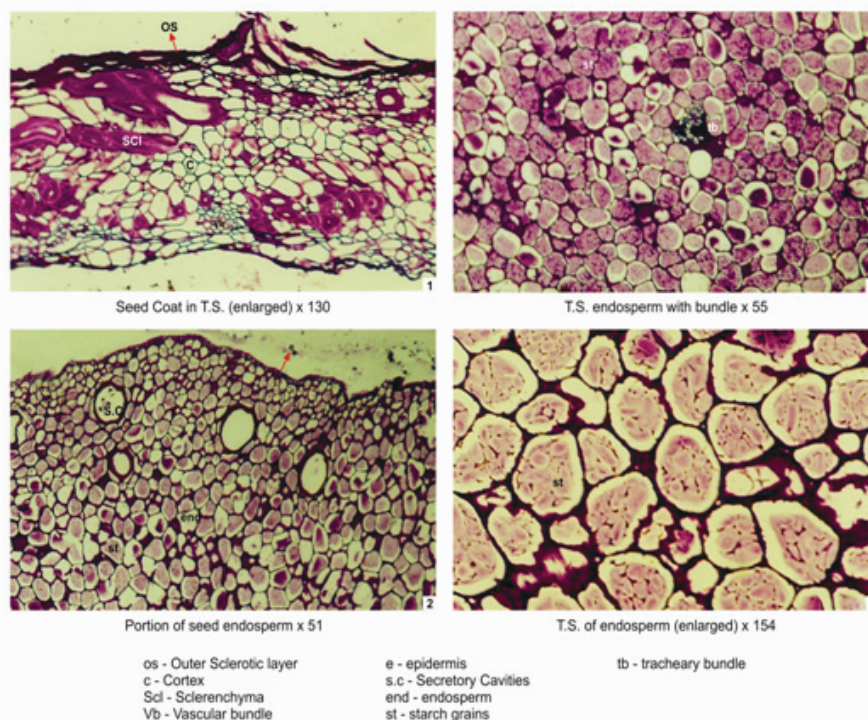
#### Pharmacognostic studies

**Macroscopy :** Seed 1.7 – 2.5 cm long and 0.9 – 1.5 cm wide, unitegmic; whitish to reddish brown, often undulated; sometimes tapering; ellipsoid to oblong, narrowed at the middle, exalbuminous, exarillate; testa slightly thick, as a sclerotic tissue separating from endosperm.

**Microscopy:** In transverse section, the seed coat is rough, papery, 2 – 3 layered and lignified, consisting of tangentially elongated cells with dark brownish black contents enclosing 20 – 40 layered cortex and at some places 10 – 12 layered. The cells of the outer layers are large, tangentially elongated, walls thick and contents dense. Cortical cells are polygonal to elongated, walls thin, contents scanty to slightly dense. The cortex is interspersed with sclerenchymatous fibers largely in groups and few isolated; cells polygonal to elongated, 22 – 97 µm (57) in diameter, while elongated cells are 108 – 227 µm (153) long and 22 – 54 µm (37) wide. Vascular bundles are present towards inner epidermis of seed coat, consisting of few xylary elements enclosed by phloem. The inner epidermis is 1-layered and is the innermost layer of the seed coat (Fig.1).

The endosperm consists of 1-layered epidermis and a few layers of smaller, closely packed polygonal to rounded cells and abundant endosperm inside (Fig.2). The hypodermal areas of the endosperm possess large secretory cavities, which are oval to elliptic or rounded; isodiametric ones 97 – 140 µm (118) in diameter, elongated ones, 151 – 281 µm (216) long and 97 – 130 µm (113) wide (Fig.2).

The cells of the endosperm are polygonal to spherical, 43 – 108 µm (72) in diameter, walls thick, intercellular spaces small, contents dense with starch grains encapsulated in plastids (Fig.4). Some cells of the endosperm contain dark brownish black contents. Some tracheary bundles are also present in the endosperm (Fig.3).



**Table 1:** Standardisation of Raw Drug

S.No	Parameters	Quantitative values
1	Moisture content (Loss on drying at 105° C)	Not more than 5.1 % w/w
2	Total ash	Not more than 0.93 % w/w
3	Acid insoluble ash	Not more than 0.4 % w/w
4	Water soluble ash	Not more than 0.8 % w/w
5	Alcohol soluble extractive	Not less than 20.5 % w/w
6	Water soluble extractive	Not less than 18.75 % w/w
7	Extractive values in	
	a. Toluene	Not less than 1.5 % w/w
	b. Chloroform	Not less than 0.5 % w/w
	c. Methanol	Not less than 28.75 % w/w

**Table 2:** Formulation of mother tincture<sup>12</sup>

<b>Alcohol</b>	<b>86% v/v</b>
Drug strength	1/10
<b>Preparation :</b>	
<i>Syzygium jambos</i> seeds in coarse powder	100 g
Strong alcohol	900 ml
Purified water	140 ml
To make one thousand milliliters of the mother tincture	

**Table 3:** Standardisation of Mother Tincture

S.No.	Parameters	Observations
1.	Organoleptic profile	
	a. appearance	clear, non-viscous
	b. colour	Light yellowish brown
	c. odour	pleasant and aromatic
2.	Sediments	absent
3.	Weight per ml	0.85 g
4.	Total solids	2.2% w/v
5.	Alcohol content	81- 85% v/v
6.	pH	4.0 – 5.0
7.	λ max	308, 316 nm

### Powder microscopy

- Starch grains numerous, spherical to oval, oblong and elliptic; elongated 8-49  $\mu\text{m}$  (29) long and 5.5-24  $\mu\text{m}$  (15) wide; isodiametric ones 4-27  $\mu\text{m}$  (14) in diameter, mostly isolated, few in groups.
- Sclereidal fibres isolated or in groups, thick walled, lignified.
- Stone cells few, polygonal or elongated, walls thick, lignified.
- Few pieces of tracheary tissue with starch grains.
- Fragments of mesophyll tissue with tannins.
- Pieces of cuticle of seed coat.

### Organoleptic characters

- Colour - light woody brown  
Touch - smooth  
Odour - characteristic, aromatic  
Taste - acrid, characteristic

### Physico-chemical studies

The physico-chemical standards on raw drug, mother tincture preparation and its standardization, are summarised in Table 1,2 and 3 respectively. The results of TLC studies are presented in Fig.5.

### Discussion

#### Pharmacognosy

In *Syzygium jambos* the endosperm is composed of 1-layered epidermis followed by underlying smaller closely packed polygonal to rounded cells. Large secretory cavities, oval to elliptic or rounded, 97-140  $\mu\text{m}$  (112) in diameter and elongated ones 151-281  $\mu\text{m}$  (216) long and 97-130  $\mu\text{m}$  (113) wide occur in the hypodermal areas (Fig.2).

The endosperm is abundant and polygonal to spherical cells 43-108  $\mu\text{m}$  (72) in diameter with thick walls and densely filled starch grains. Some cells also possess dark brownish contents (Fig.3,4). The salient pharmacognostic features along with powder microscopic and organoleptic characters presented will be helpful in authentication of the drug.

### Physico-chemical

Preliminary phytochemical screening reveals the

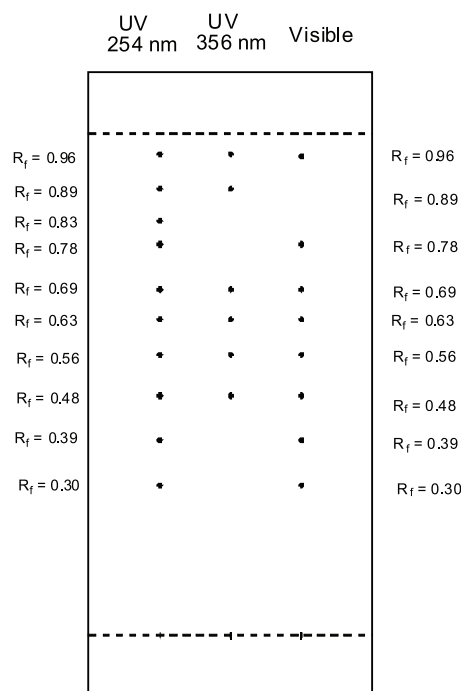


Fig.5 : Results of TLC studies

presence of tannins, alkaloids and traces of fixed oils. The values obtained for the raw drug and finished product as summarised in tables 1-3 are recommended as standards of the drug.

The results of the TLC studies given in Fig.5 are analyzed. The R<sub>f</sub> values of the spots observed under UV light (254 nm) are 0.3, 0.39, 0.48, 0.56, 0.63, 0.69, 0.78, 0.83, 0.89 and 0.96 (all light blue); under fluorescent light (366 nm) are at 0.48 (blue), 0.56 (blue), 0.63 (green), 0.69 (red), 0.89 (blue) and 0.98 (blue) whereas, in day light are at 0.3 (brown), 0.39 (brown), 0.48 (brown), 0.56 (brown), 0.63 (green), 0.69 (brown), 0.78 (brown) and 0.95 (yellow). Among the separated spots, six of them occur uniformly in different wave length. The observed chromatographic behaviour may be taken as characteristic standards for identification of the drug.

### Acknowledgement

The authors are thankful to Dr. Alok Kumar, Director General I/C, Central Council for Research in Homoeopathy, New Delhi for facilities and encouragement; to Dr. Uday Kumar, Deputy Director, Pathology Division, National Institute of Nutrition, Hyderabad for microtome facility.

### References

1. Anonymous. Wealth of India – Raw Materials, Vol.10. Publications and Information Directorate (CSIR), New

- Delhi 1982:104-5.
2. Boericke, W. Pocket Manual of Homoeopathic Materia Medica. (Repr.Ed.), B. Jain Publishers, New Delhi, 1988.
  3. Allen T.F. The Encyclopedia of Pure Materia Medica. (Repr.Ed.), B. Jain Publishers, New Delhi, 1982.
  4. Hering C. The Guiding Symptoms of our Materia Medica. Repr. Ed. Vol.6,p 291, B. Jain Publishers Pvt. Ltd., New Delhi, 2000.
  5. Slowing K., M. Shollhuber, E. Carreters and A. Villas. Flavonoid glycosides from *Eugenia jambos*. *Phytochemistry*, 1994; V. 37: 255. (1994).
  6. Chakravarty A.K., B. Das, T. Sarkar, K. Masuda and K. Shiojima. Ellagic acid derivatives from the leaves of *Eugenia jambos* L. *Indian J. Chem.* V. 37 B. 1998:1316.
  7. Jayasinghe, U.L.B., R.M.S. Ratnayake, M.M.W.S. Nedawala and Y. Fujinota. Dihydrochalcones with radical scavenging activity from the leaves of *Syzygium jambos*. *Natural Product Research*, V. 21(6), 2007 551-54.
  8. Johansen, D.A. *Plant Microtechnique*. McGraw Hill Book Co. New York, 1940.
  9. Anonymous. *Homoeopathic Pharmacopoeia of India*, Vol.1. Controller of Publications, Ministry of Health and family Welfare, New Delhi, 1971.
  10. Stahl, E. *Thin Layer Chromatography, a Laboratory Hand book*, Springer-Verlag, Berlin, 1969.
  11. Wagner, H. , Bladt, S. and E.M. Zgainski. *Plant Drug Analysis, A Thin Layer Chromatography Atlas*. Springer-Verlag, Berlin, Heidelberg, 1996.
  12. Anonymous. *Homoeopathic Pharmacopoeia of India* Vol.6. Controller of Publications, Ministry of Health and family Welfare, New Delhi, 1990.

