Standardisation of *Ammi visnaga* L. Fruit – A homoeopathic drug

P. Padma Rao[1], P. Subramanian[1], P.R. Reddy[1] and H.C. Gupta[2]

[1]Drug Standardisation Unit, O.U.B. 32, Road No. 4, Habsiguda, Hyderabad -7.
[2]Central Council for Research in Homoeopathy, New Delhi – 58.

Ammi visnaga L. is an annual herb belonging to the family Apiaceae. The dried ripe fruits of this plant are powerful bronchial antispasmodic, cures asthma, besides a strong photosensitiser. The fruit is small, 2 – 2.5 mm in diameter, oval or ellipsoid to lanceolate; each mericarp in transection appears as a pentagon; vittae are present in the secondary ridges; primary ridges possess glandular lacunae; endosperm is enclosed by a testa; aleurone grains are present in the endosperm besides a few sphaeraphides. The powder microscopic and organoleptic characters are provided. Physico-chemical parameters of raw drug viz., extractive values, ash values, formulation, besides weight per ml., total solids, alcohol content along with Thin Layer Chromatographic (TLC) and Ultra Violet Spectroscopic (UV) studies have been undertaken for mother tincture.

Keywords: *Ammi visnaga* Linn; fruits; homoeopathy; standardization; pharmacognosy; physico-chemical studies

Introduction

Ammi visnaga L., commonly known as 'honey plant' or 'tooth pick fruit' in English and 'khella' locally is an annual herb belonging to family Apiaceae. It is indigenous to Nile delta and Mediterranean region and cultivated in Latin America and India. The dried ripe fruits are medicinally used. The plant is glabrous, 50 – 80 cm high, much branched with leaves ovate in outline. Umbels mainly terminal; bracts 1 or 2 pinnatisect, as long as or longer than rays; rays are 150 cm; slender and patent in the flower, becoming erect, thicker and indurate in the fruit. Bracteoles are subulate; pedicels are erect, stout and rigid in the fruit. The fruit is 2 – 2.5 mm in diameter.[2,9]

The fruit is a powerful bronchial antispasmodic and frees from allergic bronchial asthma. It is a strong photosensitiser and on prolonged use is hepatotoxic.

Chemically khellin (2-methyl-5,8-dimethoxy furanochromone),visnagin and khelolglucoside, are the active constituents, of which khellin is most important. Other constituents are carvacrol, khellol, palmitic acid, visnadin, khellinol, ammiol, visammiol, isorhamnetin, kaempferol and terpineol.[1,3,9]

The earlier studies on *Ammi visnaga* L., pertaining to pharmacognostic and phytochemical parameters are available through the works of Kamil et al. [1] However, standardization studies in homoeopathic perspective is never done. Hence the authors have carried out detailed pharmacognostic and physico-chemical studies as per the protocols suggested by Central Council for Research in Homoeopathy (CCRH).

Material and Methods

Macroscopical and Microscopical studies

The fruits of *Ammi visnaga* L. were supplied by Survey of Medicinal Plants and Collection Unit of CCRH, Nilgiris, Tamil Nadu. The fruits were boiled and fixed in F.A.A. (Formaldehyde-acetic acid-alcohol) and processed for microtomy following Johansen.[4] Subsequently, sections (T.S. and L.S.) were obtained at 6-8 µm thickness on Leica RM 2155 microtome. The sections were stained with crystal violet and basic fuchsin and mounted in Canada balsam. The powder microscopic characters were studied by boiling powdered drug in distilled water, stained in saffranin and mounted in glycerine. Photomicrography was done with Olympus CH – 2 trinocular microscope.

Physico-chemical studies

The air dried sample of fruits are coarsely powdered and was subjected to determination of moisture

* *Address for Correspondence:*
Dr. P. Padma Rao, Drug Standardisation Unit, O.U.B. 32, Road No.4, Hyderabad -7.

content (loss on drying at 105° C), total ash, water soluble ash, acid insoluble ash, extractability in water and alcohol for raw drug and weight per ml, total solids, alcohol content for finished product. The above parameters were determined as per procedure given in Homoeopathic Pharmacopoeia of India[5]. The mother tincture was prepared as per H.P.U.S.[6]

Mother tincture (Alcoholic extract) was studied for its physico-chemical, chromatographic and spectroscopic absorbance. All chemicals and solvents used were of analytical grade. Silica gel 'G' (E Merck, India) was used for Thin Layer Chromatography[7,8] (TLC) and work was carried out at room temperature. The TLC plate was developed using chloroform-ethanol (98.5: 1.5 v/v) as mobile phase; 5 % methanolic KOH was used as spray reagent. The mother tincture was diluted with methanol and UV spectroscopy was done. The maximum absorption is recorded.

Observations and Results

Macroscopy

Fruits small, 2-2.5 mm in diameter, yellowish brown, ellipsoid, broadly ovoid, lanceolate to pear shaped, double achenes (cremocarp) split into two simple achenes. Each mericarp is glabrous, greyish brown with five prominent primary ridges and with faint secondary ridges.

Microscopy

In transverse section the fruit appears as a pentagon with a broader commissural face as an attaching side of two mericarps. The outermost 1-layered epidermis consists of polygonal to tabular cells covered by a striated cuticle with some papillae in between. The mesocarp or cortex is parenchymatous having vascular strands of a few xylary cells enclosed by phloem occurring in the ridges. The cells of the cortex are polygonal to rounded and elongated, often with dense contents. Glandular lacunae often with yellowish contents are present between the epidermis and vascular strands in the primary ridges. The hypodermis in secondary ridges is made up of club shaped epithelial parenchymatous cells radiating from secretory canals or vittae, having dark brown contents. The vittae, are small, narrow, oblong to elliptic or tangentially elongated with thick walls. The inner epidermis or endocarp is conspicuous and consists of a layer of large polygonal to tabular and elongated cells enclosing the testa (Figs.1&2)

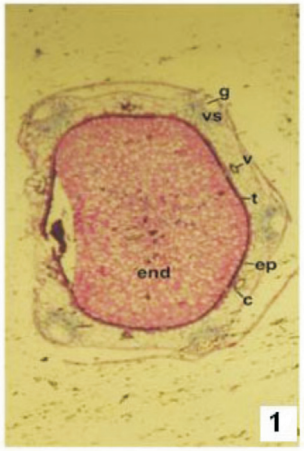

Fig.1: T.S. of mericarp X 66.

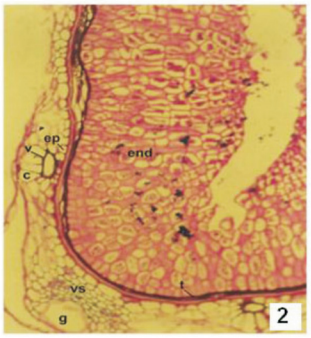

Fig. 2: T.S. of mericarp (a portion enlarged) X 151.

Abbreviations

c= club shaped cells; end = endosperm; ep = endocarp; g = glandular lacunae; t = testa; v = vittae; vs = vascular

The testa is 2-layered, made up of thin-walled tabular cells with yellowish to brown contents. A layer of cuticle covers the testa. The endosperm consists of polygonal to rounded parenchyma cells containing aleurone grains. Few sphaeraphide containing idioblasts are present in the endosperm (Figs.1&2).

Powder microscopy

Pieces of elongated hypodermal cells; pieces of parquetry cuticle; tracheary cells with helical thickenings and attached fibers; secretory canals or vittae with attached parenchyma; numerous aleurone grains; thick-walled fibres; pieces of testa with brown contents; broken crystals of calcium oxalate, few.

Organoleptic characters of powder

Colour	-	greenish yellow
Touch	-	coarse, granular
Taste	-	bitter, tingling
Odour	-	aromatic

Physico-chemical studies

The determined data under the physico-chemical study for the raw drug is summarized in Table 1 and that of mother tincture preparation and standardisation in Table 2 & 3 respectively.

Thin Layer Chromatography (TLC)

TLC was carried out on plates prepared from a slurry of Silica gel 'G' (E Merck, India) and Chloroform-Ethanol (98.5:1.5 v/v) was the developing solvent system, the spots were detected by spraying the plate

Table 1: Standardisation of Raw Drug

S.No	Parameters	Quantitative values
1	Moisture content (Loss on drying at 105° C)	Not more than 8.9 % w/w
2	Total ash	Not more than 9.93 % w/w
3	Acid insoluble ash	Not more than 1.15 % w/w
4	Water soluble ash	Not more than 3.25 % w/w
5	Alcohol soluble extractive	Not less than 5.5 % w/w
6	Water soluble extractive	Not less than 11.5 % w/w
7	Extractive values in	
	a. Toluene	Not less than 6.1 % w/w
	b. Chloroform	Not less than 4.0 % w/w
	c. Methanol	Not less than 8.5 % w/w

Table 2: Formulation of mother tincture (Percolation technique was used[5])

Alcohol	65 % v/v
Drug strength	1/10
Preparation :	
Ammi visnaga fruits in coarse powder	100 g
Strong alcohol	680 ml
Purified water	350 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	brownish yellow
	c. odour	Strong and aromatic
2.	Sediments	absent
3.	Weight per ml	0.9 g
4.	Total solids	1.56 % w/v
5.	Alcohol content	61- 65 % v/v
6.	pH	5.0 – 6.0
7.	λ max	208, 249 & 326 nm

Table 4 : Chromatographic results of *Ammi visnaga*

Solvent system	Detecting agent	No. of spots	Rf values	Colour of spots
Chloroform : Ethanol (98.5 ;1.5 v/v)	5% methanolic KOH	4	0.88 0.94 0.38 0.31	Pink Light yellowish brown do do

with 5% methanolic KOH. The results of TLC studies are presented in Table 4.

Discussion

The salient macroscopic and microscopic features of the fruits studied confirm earlier studies. The powder microscopic features and organoleptic characters along with the anatomical studies are diagnostic and establish the standards for the drug.

The values of total ash, water soluble ash and acid insoluble ash are in agreement with the values reported earlier. However, loss on drying, extractability in water and alcohol and different solvents falls in the acceptable range. The methodology for preparation of mother tincture and its standardization are presented in Tables 2 & 3, which are specific to Homoeopathic pharmacopoeial standards and will supplement substantially. The results of TLC studies given in Table 4 reveals four distinct spots and UV spectrophotometric study exhibits (maximum absorption) three prominent peaks which can be taken as characteristic standard for the drug.

The determined physico-chemical data, macro and microscopical characters and methodology employed in the study will help in identification, authenticity and ensures quality, purity and efficacy of the drug.

Acknowledgement

Dr. Alok Kumar, Director General I/C, Central Council for Research in Homoeopathy, New Delhi, for his kind support and encouragement.

References

1. Kamil M, Sheikh M O El, Ahmad F, Katheer, P Al and Naji M A. Pharmacognostic and phyto-chemical standardization of *Ammi visnaga Hamdard medicus.* V.51(1), 125-137, (2008).
2. Anonymous. The Wealth of India, Raw Materials. Publications and Information Directorate (CSIR), New Delhi. Vol. 1, p.226. (1985).
3. Nielsen B E. Coumarin patterns in the Umbelliferae.In. The Biology and Chemistry of Umbelliferae. Ed.Heywood,V.H. Academic Press, London. (1971).
4. Johansen D A. Plant Microtechnique. MacGraw Hill Book Co., New York. (1940).
5. Anonymous. Homoeopathic Pharmacopoeia of India. Vol.1, Controller of Publications, Ministry of Health & Family Welfare, New Delhi. (1971).
6. Anonymous. United States Homoeopathic Pharmocopoeia Convention. (1993).
7. Stahl E. Thin Layer Chromatography, A Laboratory Handbook. Springer- Verlag, Berlin. (1969).
8. Wagner H and Bladt S. Plant Drug Analysis. A Thin Layer Chromatography Atlas. Springer-Verlag, Berlin, Heidelberg. (1996).
9. Trease G E and Evans W C. Pharmacognosy. 11th Ed. Baillere Tindall, New York. (1978).