Journal of Experimental Sciences Vol. 1, Issue 11, Pages 20-24 [2010]
www.jexpsciences.com

Regular Article

Development of Quality Standards of *Ammi majus* L. Fruit

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ABSTRACT: *Ammi majus* Linn (Apiaceae) is an erect branching annual herb, cultivated in northern part of India mainly Jammu and Kashmir. It is commonly used in day to day life. In present investigation an attempt has been made for the pharmacognostical standardization and evaluation of Ammi majus fruit. The pharmacognostical evaluation comprises of detailed macroscopy, powdered microscopy, fluorescence analysis and physical constants such as ash and extractive values. The data obtained in present study will serve as valuable tool for identification, authentication and detection of adulterants, standardization and quality control of the drug. The developed technique will also be useful for the standardization of formulations containing *A. majus*.

Key words: Ammi majus, Extractive values, Ash values

Introduction

Ammi majus Linn. (Apiaceae) commonly known as AATRILAL, Bishop's weed or Greater Ammi indigenous to Egypt and widely distributed in Mediterranean region, Abyssinia, Europe, West Africa, freely grows in the Nile Delta region. It is cultivated in various parts of India, where the plant was first introduced from Egypt at Forest Research Institute, Dehradun in 1955. Since then its experimental cultivation has been tried in several parts of the country. The fruits are valued in indigenous medicine and have been employed for long time in folklore therapy for the treatment of leucoderma, vitiligo, diuretic, emmenogogue, and abortifacient and blood purifier [1]. The preliminary phytochemical investigation of *A. majus* showed presence of certain biochemical constituents namely coumarins, furocoumarins, flavonoids that are responsible for various pharmacological activities including leucoderma and vitiligo. The compositions of constituents are affected by various geographical parameters, improper collection, storage condition that can lead to deterioration of the raw material [2,3,4] hence it was essential to standardize the fruit of *A. majus* for the establishment of quality and identity profile of the drug for the purpose of safety monitoring and overall quality assurance of the industrially as well as commercially important drug i.e. *A. majus*. Since there is no report in literature regarding the standardization of *A. majus* fruit, therefore, in the present investigation an attempt has been made to standardize *A. majus* fruit by using macroscopy and microscopic characters, powder microscopy, fluorescence analysis, physico-chemical values.

A. majus is an erect branching annual herb, attaining height up to 1.5-2.0 m with whitish tap-roots; stem erect, slender, glabrous with fine longitudinal striations; leaves alternate with long petiole, pinnately divided, lobes oblong, acutely serrate; inflorescence a compound umbel, the involucral bracts generally divided and about 13 in number, bracteoles of the involucre about 8 in number, primary ray of umbel sometimes 5 cm long; slender, secondary rays 2-5 cm long; flower whitish actinomorphic or zygomorphic, bisexual, pentamerous and bracteate; calyx teeth obsolete or small; petals obovate with an inflexed point, the exterior ones frequently longer; stamens epigynous; ovary inferior, 2-celled, stigma capitate; fruit a cremocarp with slender prominent ribs, laterally compressed, oblong, smooth, 1.5-2.0 x 1.0 or less mm in size mericarps separated by carpophore and bearing a stylopodium at its apex; seed small pendulous and albuminous [2,4].

The drug has been the subject of a good deal of chemical and some pharmacognostical investigation, due to the greater interest in its active constituents useful in various ailments. Certain phytoconstituents like ammoidin [5], ammajin from fruits of Pakistani species [6], furocoumarins from the seeds and fruits [7,8], flavonoids [9], iso-pimpinallin [10], isoimperatorin [11], some nonhydroxylic coumarins [12], ammajin and marmasin [13], ammirin and alloimperatorin [14], khellin and visnagin [15].essential oils [16] are reported.

Method for identification of active principles of *Ammi majus* by absorption of spectra was also developed [17].

Material and Methods

Chemicals and reagents

All the chemicals and reagents used were of analytical grade, purchased from Sigma chemical co. (St Louis, MQ, USA) and Merck (Darmstadt, Germany). Fruits of *A. majus* were collected from campus of Hamdard University, New Delhi, India, (July -2007), which was identified by Taxonomist (Professor M.P. Sharma), Department of Botany, Hamdard University New Delhi. The voucher specimen was deposited in Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard .

Morphological studies

The morphological studies were carried out for shape, size, colour, odour, taste and fracture of the *A. majus* fruit.

Microscopic studies and powder analysis

The transverse section of fruit was prepared by standard method. Slides of powdered fruit material were also prepared and studied. Microphotography on different magnifications was carried out with motic microscopic unit. Polarized light was used for the study of crystals, starch granules and lignified cell.

Physicochemical Standardization

The various physico-chemical values of fruit such as ash values, extractive values were determined according to the Pharmacopoeial method which are given in Table 1,2.

Fluorescence Analysis

The fluorescence nature of powder drug was analyzed and the observations with different chemicals were also carried out and recorded which are given in Table 3,4.

Results and Discussion

Macroscopical evaluation

The fruit of *A. majus* were subjected to macroscopical examination and observations were recorded. The proper examination of the fruit was carried out under sun light and artificial source similar to day light. The fruits are entire cremocarps (schizocarps) and separate mericarps. Pedicel is usually attached with fruit. The fruits are glabrous, cylindrical to oblong-obovoid, 2.5 - 3.0 mm long and 1 mm or less wide, yellowish-brown in colour. Stylopods on the tip of the fruit are bifid and free ends of which curve along the dorsal sides. The dorsal side of the mericarp is convex and consists of five prominent, longitudinal yellow ridges running from base to apex and four dark brown furrows. The commissural surface is almost flat with central line, *i.e.*, carpophore extending from base to apex. The taste

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of the drug is extremely pungent but slightly bitter and possesses a characteristic terebinthinate odour.

Whole Plant

Fruits

Microscopical evaluation

The slides of T.S of fruit of plant were prepared and subjected to microscopical examination. The histology was examined and the observations were recorded. The T.S of *A. majus* fruit **[Plate 1(Fig.1, 2)]** showed the fruit is almost pentagonal on outline with orthospermous seed. There are five vascular strands, which are found below each primary ridge alternating with four secondary ridges. There are four-six vittae and each lies between two primary ridges and below the secondary ridges, which are 60–80 x 20–40 μ in size. On the outer side of each vittae is a radiating club-shaped cells.

Epidermis (Epicarp)*:* The epicarp is single layered and consisted of sub-rectangular polygonal and slightly tangentially elongated cells, the outer walls of which are convex and these cells are measuring 13–72 x 11–18 μ in size. The outer wall in many epicarp cells is plain, which in others, especially on the ridges, slightly protrudes out to from short papillae, and each cell generally contains single rosette of calcium oxalate crystal, varying in size. Stomata are mostly spherical to elliptical and are of cruciferous or caryophyllaceous type.

Mesocarp*:* The mesocarp consists of a large number of tangentially elongated, thin walled parenchymatous cells in several rows, measuring 30-71 x 11-21 μ in size. Towards the outer side of the vittae, *i.e.*, towards epicarp, the cells of the mesocarp are radially elongated, comparatively longer than the other mesocarp cells, which is a characteristic of the genus. The inner mesocarp consists of few layers of slightly thickened (sclerenchymatous) yellowish brown to reddish brown cells. The vittae extends the whole length of the fruit throughout the mesocarp. The vittae are found to be oval and yellowish brown in colour. The vascular bundles (primary ridges) are nearly circular in outline with small phloem patches on either side of the outer part of the xylem.

Endocarp*:* The endocarp is single layered and consisted of narrowly elongated cells having a perquetry arrangement being arranged in groups of four to nine parallel narrow cells. In transverse section the endocarpal cells appear as tangentially elongated rectangular cells, showing here and there distant partition and they are 36-89 x 4-6 μ in size.

Endosperm*:* The endosperm is generally composed of polygonal, thick walled cells 16-60 x 9-30 μ in size, containing fixed oils and numerous small oval or rounded aleurone grains ranging from 4 to 12 μ in diameter. The aleurone grains usually contain one or rarely two-minute rosettes of calcium oxalate crystals. The embryo is small and lies almost surrounded by endosperm. The raphe bundle is situated in centre towards the commissural side and surrounded by parenchymatous tissue.

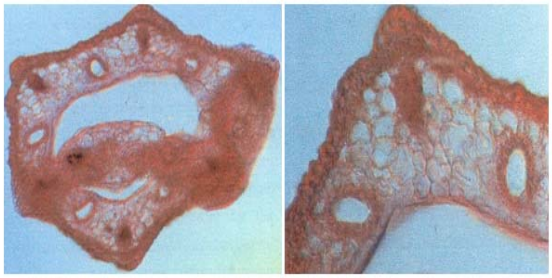
Powder microscopy

Powder of the crude drug was yellowish brown, coarsed free flowing. The taste was slightly bitter but externally pungent and had characteristic terebinthinate odour. Small amount of powdered material (sieved through 40 mesh) was placed on microscopic slide; mixed with few drops of 40% w/v aqueous chloral hydrate and heated gently under Bunsen burner. After adding few drops of 1% alcoholic phloroglucinol, it was warmed by mixing one drop of concentrated hydrochloric acid. The slides were mounted in glycerine and observed under microscope. The powder characterized microscopically by the presence of fragments of epicarp with occasional stomata, endospermic cells, parquetry cells of endocarp, polygonal mesocarpic cells; fragments of brown vittae adhering to the cells of mesocarp, narrow annular and spiral vessels, sclerenchymatous fibres of the vascular bundles, some club shaped cells and few tracheids. Druses and aleurone grains are abundant.

Maceration

Maceration of the fruits was carried out [18]. The macerated tissues were studied in 1% saffranin in alcohol and mounted in glycerine to examine non-protoplasmic cellular contents, *e.g.* cells or fragments of number of endospermous cells and large number of endospermous cells and spiral or annular vessels, sclerenchymatous fibres and tracheids.

Plate-1



X400 X400

Plate-2

Fig.1- Diagrammatic figure of atransverse section of a mericarp

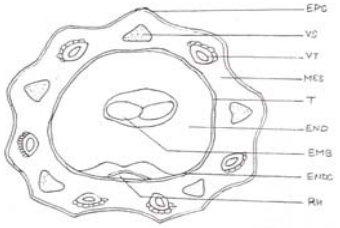


Fig.2- T.S of a mericarp through a part showing details of structure

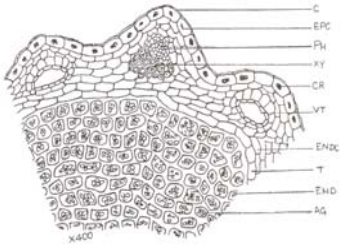
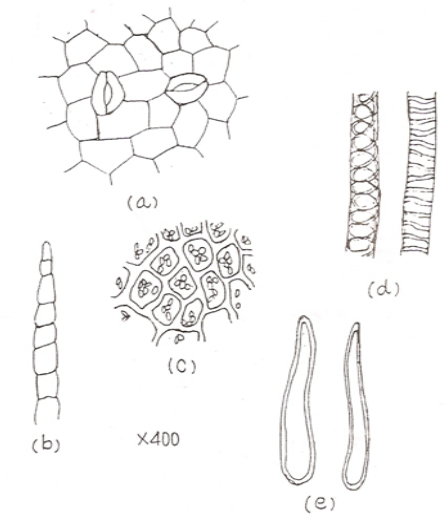


Fig.3- Maceration
(a) Surface view of cruciferous stomata
(b) Vittae in surface view
(c)Surface view of endosperm
(d) Spiral and annular vessels



Abbreviations:
AG = Aleurone grain; C = cuticle; CR = Rosette crystal of calcium oxalate; EMB = Embryo; END = Endosperm; ENDC = Endocarp; EPC = Epicarp; MES = Mesocarp; PH = Phloem; RH = Raphe; T = Testa; VS = Vascular supply; VT = Vittae; XY = Xylem

Table -1 Ash Values of *A. majus* Fruit

S.No.	Determinants	Values in Percentage
1.	Total ash	6.42
2.	Acid insoluble ash	0.05
3.	Water soluble ash	6.38

Table-2 Extractive values of *A. majus* fruit

S. No.	Extractive Solvents	Values in Percentage
1.	Petroleum ether (60-80°)	2.84
2.	Benzene	2.17
3.	Chloroform	0.46
4.	Acetone	0.82
5.	Ethanol	16.08
6.	Distilled water	16.76

Table-3 Fluorescence Analysis of Powdered *A. majus* Fruit.

S.No.	Reagents	Colour in Day Light	Observation Under UV Light		
			Modifying Colour	Colour Quality	Radiance Degree
1.	Mounted in Nitrocellulose	Yellowish green	Green	Dark	Dull
2.	1N NaOH in Methanol	Light green	Greyish green	Light	Bright
3.	Treated with 1N NaOH in Methanol and mounted in nitrocellulose	Green	Green	Dark	Bright

4.	1N Hydrochloric acid	Brown	Brown	Dark	Dull
5.	Treated with 1N HCl and mounted in nitrocellulose	Yellowish Brown	Brown	Dark	Dull
6.	1N NaOH in water	Brown	Brown	Light	Bright
7.	Treated with 1N NaOH in water and mounted in nitrocellulose	Brownish green	Green	Light	Dull
8.	Diluted Nitric acid (1:1)	Orange Yellow	Brown	Light	Dull
9.	Diluted Sulphuric acid (1:1)	Brown	Brown	Dark	Dull
10.	Powder as such	Yellowish Brown	Brown	Light	Dull

Table-4 Reaction of Powdered Fruit of *A. majus* with Different Chemical Reagents

S.No.	Chemical Reagents	Observation
1.	Conc. sulphuric acid	Reddish black
2.	Conc. hydrochloric acid	Dark brown
3.	Conc. nitric acid	Orange yellowish
4.	Pot. hydroxide solution (aqueous) (5%)	No change
5.	Sodium hydroxide solution (aqueous) (5%)	No change
6.	Ferric chloride (aqueous)	Bluish green
7.	Iodine solution	No change
8.	Picric acid	No change
9.	Acetic acid glacial	No change
10.	Powder as such	Yellowish brown

Discussion

The drug 'aatrilal' has been in use in Unani System of medicine since long for the cure of leucoderma (vitiligo). Fruit and seeds are the main source of the drug. The drug is reported to be diuretic, emmenogogue, abortificient, blood purifier and anti-inflammatory [1,2,3,19]. The drug 'aatrilal' has been attributed to *Ammi majus* Linn. (Family: Apiaceae) in most of the books on medicinal plants, but it has been equated with an Acanthaceous plant *Peristrophe calyculata* Nees. *Peristrophe* can easily be distinguished from *A. majus,* as its fruits are not cremocarp [3].

The fruits of an allied species *A. visnaga* are sometimes substituted for the genuine drug. Epicarp of *A. majus* and *A. visnaga* were inspected by SEM and a number of microcharecters to distinguish the two species were suggested [20]. Detail macro and microscopic characters of the fruit and seeds were studied in the present investigation with a view to bring out some distinguishing characters of the fruits and identifying it when adulterated with other similar types of umbelliferous fruits. Other pharmacognostical parameters such as fluorescence analysis of the powdered drug, ash values were also taken into account. The macro and microscopic features of the fruits of *A. majus* reported in the present investigation are almost congruent with the earlier reports. Fruit anatomy is used as the main criterion for the classification of Umbellifereae because of the distinctiveness and consistency of fruit structure [21]. Therefore, the morphological and anatomical details investigated in the present study will help in distinguishing *A. majus* fruit from its adulterants and substitutes. Since the fruits of *A.majus* and *A. visnaga* are similar and are reported to often occur admixed, their botanical identification is rather problematical [4].

Slight variation was found in other evaluative parameters (flouresence analysis and physical constant values) from those reported for *A.majus* by [22]. This may be due to variation in maturity, time of collection and storage condition of the fruits.

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