

Traditional claims to Scientific Evidence: A Analysis of *Trachyspermum ammi*

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ABSTRACT

Background: *Trachyspermum ammi* (Ajwain) is a medicinally significant plant belonging to the Apiaceae family, widely cultivated in various regions, including India, Iran, and Egypt. **Objectives:** This study provides a detailed Pharmacognostic evaluation of ajwain through macroscopic, microscopic, powder microscopy, histochemical, and preliminary phytochemical analyses, along with standardization parameters for crude drug validation. **Materials and methods:** Ajwain fruits were collected and authenticated by a certified botanist. Macroscopic and microscopic analyses were conducted using digital and compound microscopes. Powder microscopy, histochemical staining, preliminary phytochemical screening, and physicochemical standardization, such as ash values, extractive values, and moisture content, were performed. **Result:** Macroscopic observations confirmed the fruit's distinctive morphological features, while microscopic studies detailed the anatomical structure, including the presence of vittae, epicarp with papillae, and oil globules. Powder microscopy further verified characteristic elements such as anomocytic stomata, fibers, annular vessels, and phenolic compounds. Histochemical tests revealed the presence of bioactive compounds like alkaloids, flavonoids, lignin, and mucilage, which contribute to its therapeutic properties. These findings substantiate the traditional medicinal applications of ajwain, particularly in gastrointestinal and respiratory treatments. **Conclusion:** The study provides essential diagnostic markers for quality control and standardization in herbal medicine, laying a foundation for future pharmacological investigations.

Keywords: Ajwain, Apiaceae, Histochemical studies, Pharmacognostical study, Standardization.

INTRODUCTION

Trachyspermum ammi (L) Sprague, or ajwain plant, belongs to the Apiaceae family, which comprises approximately 270 genera and species, and is valued for its medicinal properties as a spice^{1,2}. It is known by various names across different languages, including Ajwain in Hindi, Bishop's weed in English, Kath in Kashmiri, and Omam in Tamil.

Trachyspermum ammi is an annual herb that typically grows to a height of 60 to 90 cm, featuring a highly branched structure. Its fruit consists of two greyish-brown, flattened, oval-shaped mericarps, measuring around 2 mm in length and 1.7 mm in width³. Each mericarp possesses five distinct ridges and six vittae, with five primary ridges. The plant bears actinomorphic white flowers, which can be either male or bisexual. The corolla comprises bilobed, five-petaled flowers, with five stamens alternating with the petals. It has an inferior ovary, and its stigma is bud-shaped^{4,5}. The aromatic fruit of *Trachyspermum ammi* is ovoid and heart-shaped, resembling a cremocarp, and features a stylopodium that remains attached after maturity. The plant's pinnate leaves consist of a single terminal leaflet accompanied by seven pairs of lateral leaflets⁶.

Ajwain seeds are small, greyish, and have a pungent, slightly bitter taste. When raw, they possess a strong, peppery flavor, which mellows upon cooking. The seeds or fruits are the most commonly utilized parts of the plant, resembling cumin or caraway seeds in appearance. Traditionally, ajwain has been used

for digestive ailments, while its hot, dry fermented fruits have been applied to the chest to relieve asthma symptoms⁷. Thymol is the major bioactive constituent of ajwain oil and is used extensively to treat gastrointestinal disorders, induce appetite, and alleviate bronchial diseases. Moreover, ajwain seeds possess anthelmintic, carminative, laxative, and stomachic actions and thus are rendered useful to treat abdominal tumours, gastric colic, and piles⁸. Despite multiple studies on its phytochemical content and therapeutic applications, there remains a gap in integrative Pharmacognostic profiling, especially combining microscopy, histochemistry, and standardization measures. The taxonomical classification is shown in Table 1.

Vernacular names

Bengali- Jowan; English- Bishop's Weed; Gujarati- Ajma; Hindi- Ajwain; Kannada- Oma; Malayalam- Ayamodakam; Marathi- Onva; Punjabi- Ajwain; Sanskrit -Yamini; Tamil- Omam; Telugu- Vamu¹⁰.

Geographical distribution

Trachyspermum ammi occurs naturally in the eastern Mediterranean region and is widely cultivated in South Asia. It is widely cultivated in India, Iran, Iraq, Pakistan, Afghanistan, and Egypt. In India, particularly, it is widely cultivated in Rajasthan, Gujarat, Madhya Pradesh, Uttar Pradesh, and West Bengal because the dry environment is conducive to its cultivation^{10,11}. The plant thrives in arid and semi-arid regions, typically in well-drained soils with moderate rainfall. Due to its medicinal and culinary importance, ajwain is also cultivated in other tropical

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Table 1. Taxonomical classification (9)

Kingdom	Plantae
Sub kingdom	Tracheobionta
Phyllum	Spermatophyta
Sub-phyllum	Angiospermae, Flowering Plant
Class	Magnoliophyta
Order	Apiales
Family	Apiaceae
Genus	<i>Trachyspermum</i>
Species	<i>ammi</i> .

and subtropical regions worldwide. Image of *Trachyspermum ammi* geographical distribution was performed by using the website Plants of the World Online (POWO) by Kew (Figure 1).

METHODS AND MATERIALS

Collection and authentication of plant material

Trachyspermum ammi fruit was obtained from an herbal store at Arumbakkam, It was identified and authenticated by Dr. KN Sunil Kumar, Research Officer, Central Council of Research in Siddha, Ministry of Ayush, Arumbakkam, Tamil Nadu, India. Herbarium voucher number T16082404A. The plant parts were further processed for the coarsely powdered and kept in air tight container for experimental work.

Macroscopy

A Nikon D-5600 Digital camera was employed in photographing the external features of the test sample.

Microscopy

The sample was left in FAA fixative for over 48 hours. Sampled storage was sectioned into thin transverse sections with a sharp blade, and the sections were stained with toluidine blue¹². Transverse sections were captured under an Axiolab 5 trinocular microscope with a Zeiss

Axiocam208 color digital camera under bright field illumination. Magnifications were marked with a scale bar.

Powder microscopy

A small amount of the powdered sample was put on a microscopic slide together with a drop of 50% glycerol after clarification with a saturated chloral hydrate solution¹³. The features were analyzed using a Nikon ECLIPSE E200 trinocular microscope with a Zeiss ERc5s digital camera in incident light conditions. Photomicrographs showing the diagnostic features were taken and stored.

Histochemical testing

Plant material was processed in the standard manner¹⁴:

a. Crystals: The mounts were put in water, and one side of the cover slip was irrigated with acetic acid. On microscopic examination, the water in the coverslip was replaced by placing a strip of filter paper on the other end of the coverslip

- Air bubble formation signaled the presence of Calcium carbonate crystals

-If no air bubbles were formed, the experiment will be repeated with conc. HCl, wherein the needle formation of Calcium sulfate on dissolving the crystal confirmed the presence of Calcium oxalate

b. Fats, Fatty oils, volatile oils, and resins: 1 to 2 drops of Sudan-IV were put into the section and left for two minutes. Fatty oil material was shown by orange-red/pink/red colored globules, while red colored irregular material showed resin.

c. Starch: A few drops of a 2% solution of iodine in water produced a blue color, which indicated the presence of starch.

d. Phenolic compounds: Added alcoholic ferric chloride, one drop - bluish black colored material indicated the presence of phenolic compounds like flavonoids/tannins, etc

e. Mucilage: Added a single drop of ruthenium red - red or pink coloured material indicated mucilage.

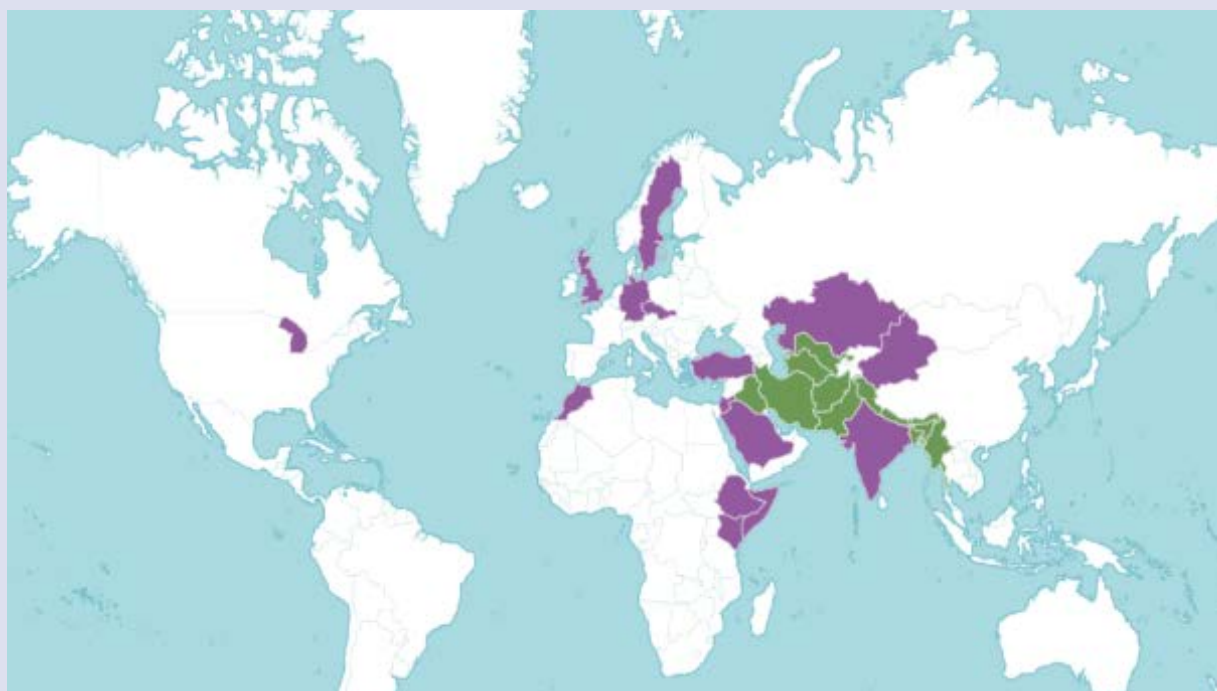


Figure 1. Geographical distribution of *Trachyspermum ammi* around the world

f. Lignified cell walls: A drop or two of phloroglucinol was put onto the section and allowed to stand for about 2 min or until almost dry. 50% HCl was added as a drop and observed under a cover glass - cell walls - pink to cherry red, indicating the presence of lignin.

g. Suberized or cuticular cell walls: A few drops of Sudan red III were added and left to stand for a few minutes, warmed gently if needed - cell walls-orange-red or red as a result of deposition of suberin or cutin on the cell wall.

h. Alkaloids: A few drops of Wagner's reagent were added - the yellow to reddish colour was apparent. **Phytochemical screening**

Trachyspermum ammi extracts were also tested for initial phytochemical screening by adopting standard protocols^{15,16}. Screening was accomplished to find evidence of alkaloids, steroids/triterpenoids, phenolics, flavonoids, saponins, tannins, and carbohydrates according to the protocol given by Harborne (1998) and Kokate (2005).

Physicochemical standardization

Various physicochemical properties of the powder, including loss on drying (LOD), pH value, total ash, acid-insoluble ash, and extractive values, were assessed following the procedures outlined in the British Pharmacopoeia and the Ayurvedic Pharmacopoeia.

RESULTS

Macroscopy

The fruit is glabrous, ovoid-conical, of two mericarps, grayish-brown, compressed, ovoid, 2 mm long, 1.7 mm broad, with 5 ridges and 6 vittae per mericarp, free or separate, 5 main ridges (Figure 1 & Table 2), fragrant and with a weakly astringent and bitter taste.

Microscopy

TS of the fruit consists of two big hexagonal mericarps joined in the middle by carpophore tissue; mericarps contain four big vittae on the dorsal side, two on the commissural side, and four big ridges on the dorsal side; 3-5 secondary oil cavities are located under each big ridge.

The epicarp is made up of elongated cells tangentially with inconspicuous striated cuticle; some of the cells of the epicarp develop papillae; the mesocarp area is made up of lignified, thick-walled,

polygonal to rectangular parenchyma cells; vittae are observed scattered in the mesocarp area, small collateral vascular bundles are observed in the mesocarp area; few schizogenous duct are also found; carpophore made up of thick-walled vascular tissue; endosperm made up of thin-walled cells with oil globules and aleurone grains (Figure 2).

Powder Microscopy

The powder is pale brown in color and shows the surface view of the epicarp with papillae; epicarp fragment with striated epidermis and anomocytic stomata; fibre; annular vessels; and oil globules; characteristic aromatic and taste slightly bitter (Figure 3).

Histochemistry

Cutin found in cells of epicarp along with oil globules; phenolic compounds are present in the endocarp region and also surrounding the vittae; alkaloid is present in few cells of the epicarp, mesocarp and endosperm; mucilage detected in the outer layer of endosperm and papillose outgrowths of epicarp; glandular trichome; lignin observed in the xylem walls of vascular bundles scattered in mesocarp; oil globules are present in endosperm cells (Figure 4).

Table 2. Morphology of *Trachyspermum ammi* fruit

Colour	Greyish-brown
Odour	Strong, pungent, and aromatic
Taste	Bitter and slightly hot
Shape	Oval, slightly curved, ridged
Size	Small, 1–2 mm in length

Table 3. Preliminary phytochemical screening of *Trachyspermum ammi* extract using different solvents

Test	Pet ether	Chloroform	Ethanol	Methanol
Carbohydrate	-	-	+	+
Alkaloids	-	+	+	+
Flavonoids	-	-	+	+
Proteins	-	-	+	+
Saponin	-	-	+	+
Tannin	-	-	+	+
Steroids	+	-	-	-

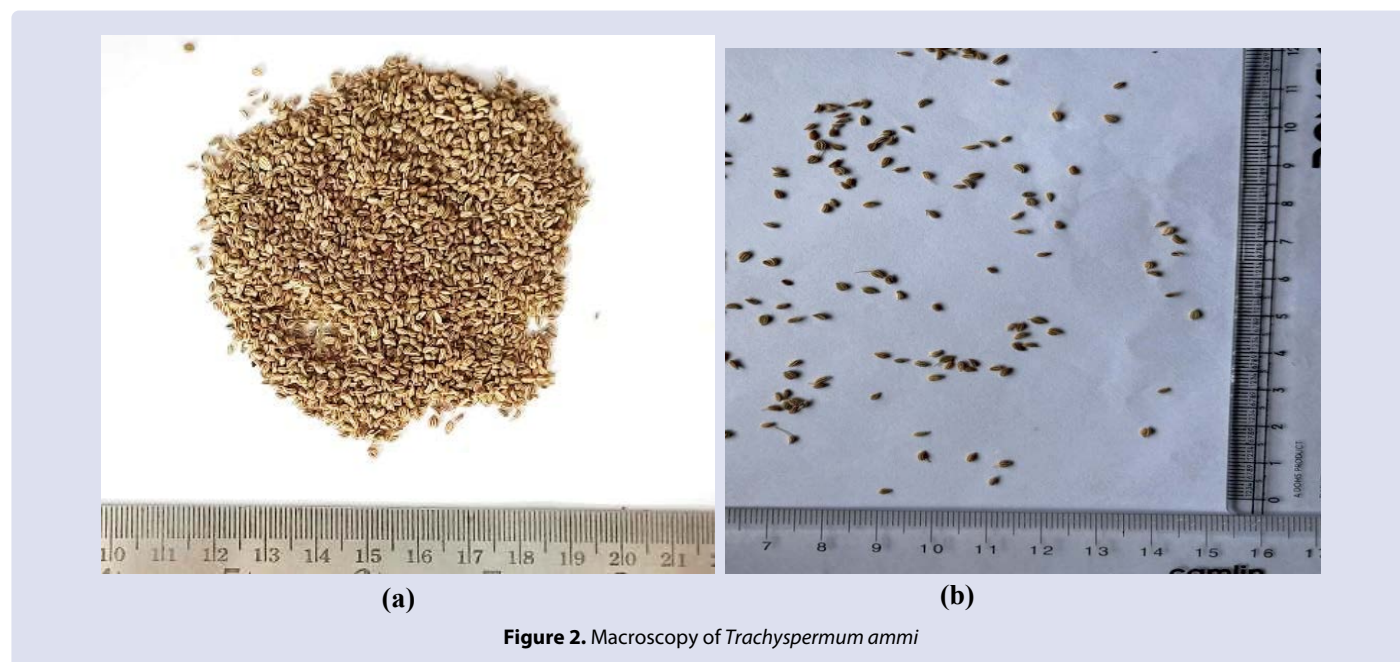
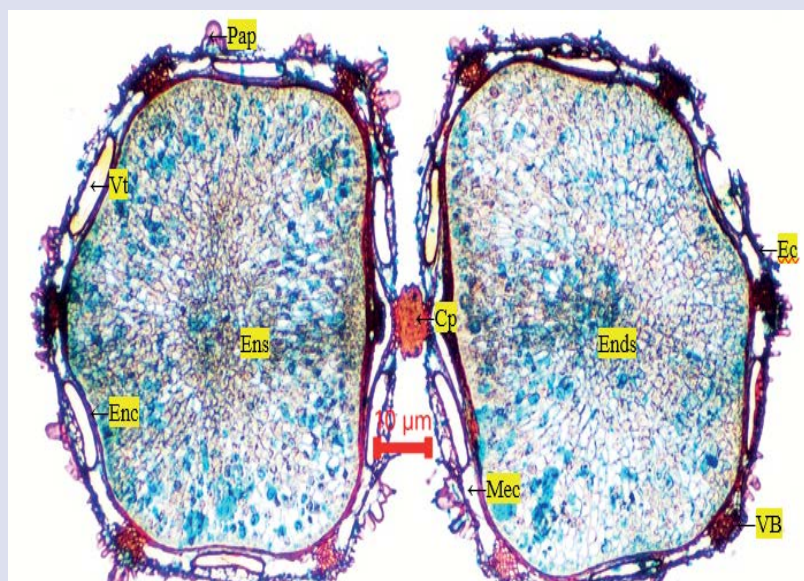
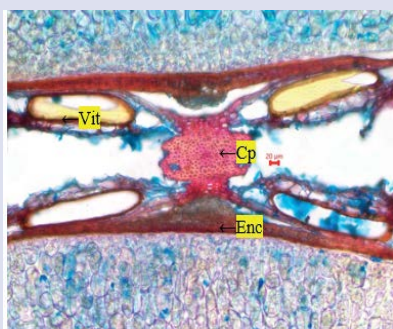


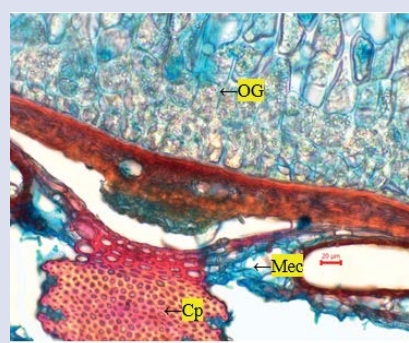
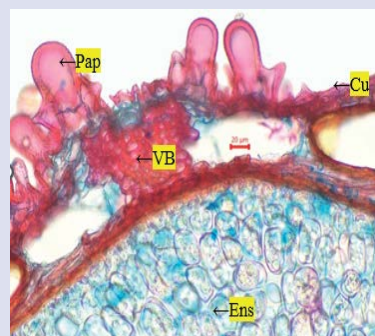
Figure 2. Macroscopy of *Trachyspermum ammi*



a) TS of fruit

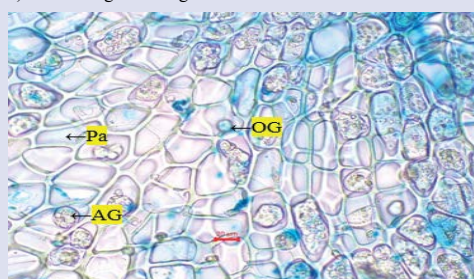


b) Outer region enlarged



c) Lateral region enlarged

d) Carphophore region enlarged



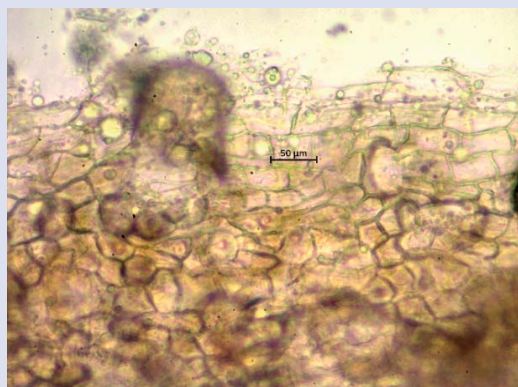
e) Endosperm region enlarged

Figure 3. Microscopy of *Trachyspermum ammi* fruit

AG – aleurone grains; **Cp** – carphophore; **Cu** – cuticle; **Ens** – endosperm; **Enc** – endocarp; **Mec** – mesocarp; **Pa** – parenchyma; **Pap** – papillae; **OG** – oil globule; **Vit** – vittae.



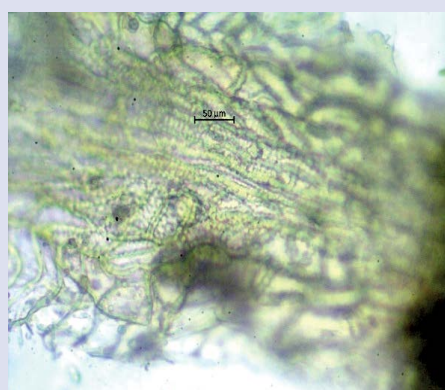
a) Pappillae



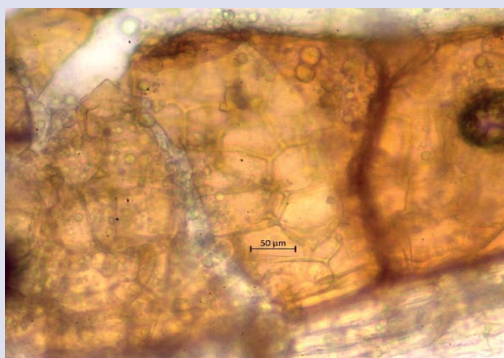
b) Epicarp in surface with papillae



c) Striated epicarp with anisocytic stomata



d) Mesocarp fragment



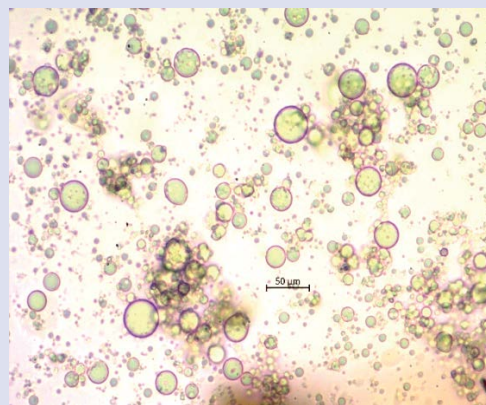
e) Vittae in surface view



f) Fragment of fibre

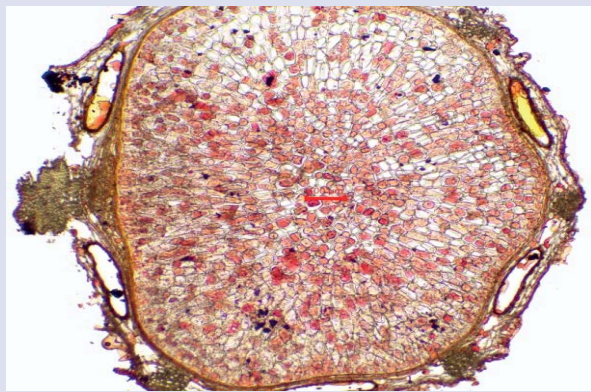


g) Pitted vessel

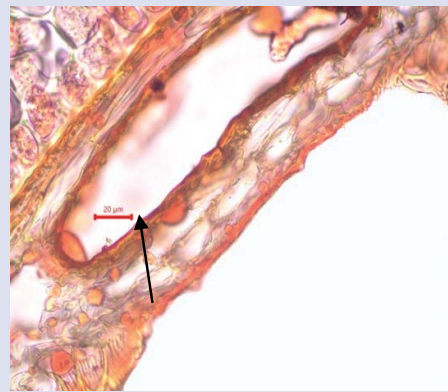


h) Oil globules

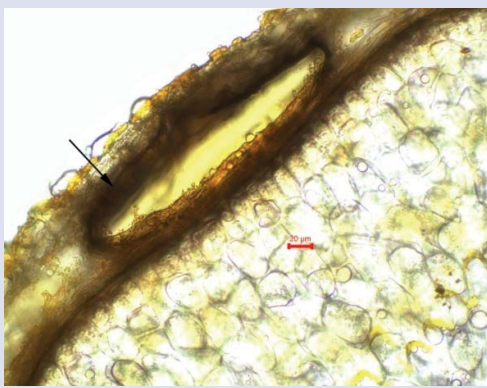
Figure 4. Powder macroscopy of *Trachyspermum ammi* fruit



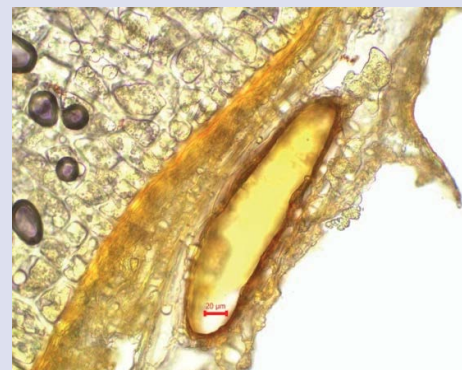
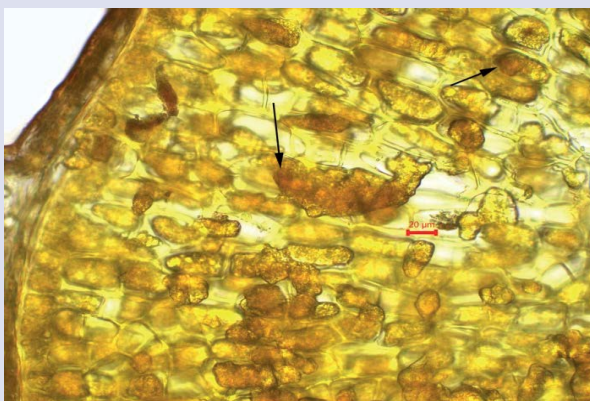
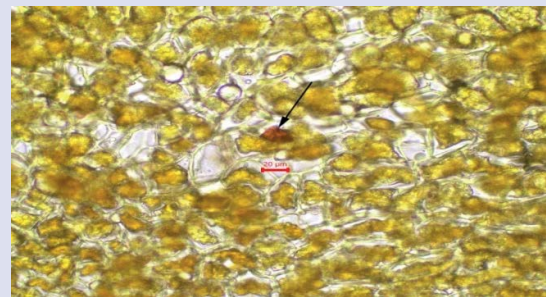
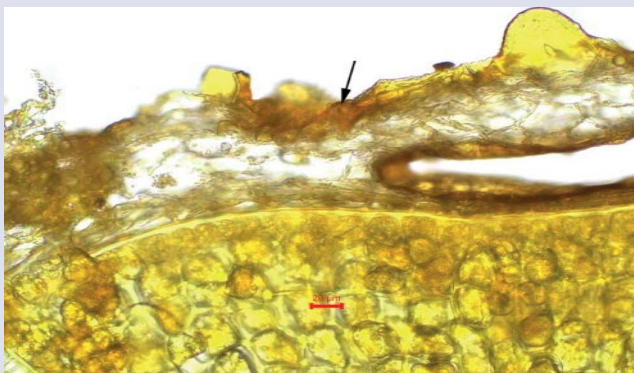
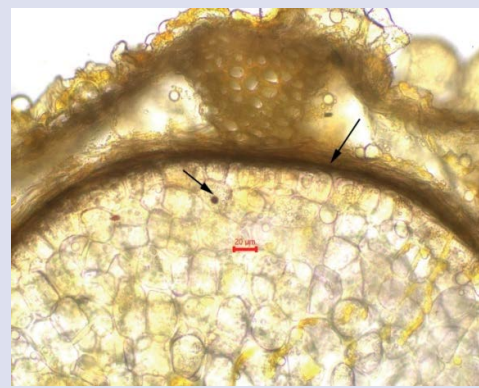
a) Cutin and oil



b) Cutin



c) Phenolic content



d) Alkaloid

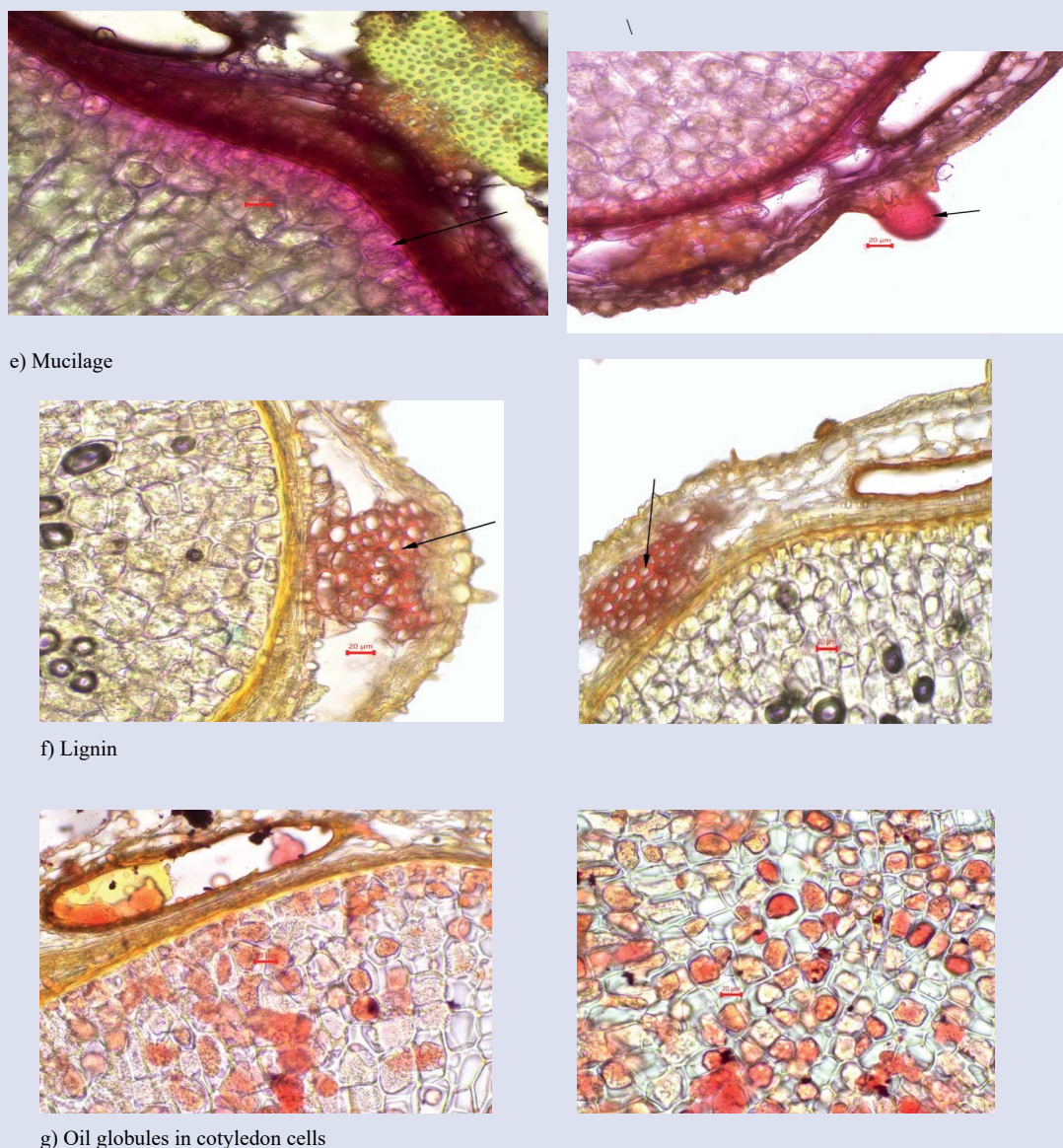


Figure 5. Histochemical study of *Trachyspermum ammi* fruit

Preliminary Phytochemical Screening

The qualitative analysis of the various extract of *Trachyspermum ammi* for the identification of phytochemical constituents such as alkaloids, flavonoids, glycosides, tannins, terpenoids, carbohydrates, and amino acids (Table 3) involves a series of tests to detect the presence or absence of these compounds in the extract.

Physicochemical standardization

Physicochemical analysis of *Trachyspermum ammi* fruit powder was subjected to Loss on drying, ash value, and extractive value are presented in Table 4.

DISCUSSION

The present work depicts a complete pharmacognostic investigation of *Trachyspermum ammi* (ajwain) through macroscopic, microscopic, powder microscopy, and histochemical analysis. The findings validate the typical morphological, anatomical, and chemical characteristics

responsible for its identification and clinical application. It is interesting to note that macroscopic analysis of plant material is usually prone to subjective error, and substitutes or adulterants can closely resemble the true material. Macroscopic findings thus need to be validated and confirmed by microscopic and physicochemical studies.

Microscopic examination of crude drugs is essential in the identification of ground or powdered drugs.

Macroscopy and Microscopy

The macroscopic observations revealed that *Trachyspermum ammi* fruits are small, grayish-brown, and ovoid-conical in shape, with five ridges and six vittae per mericarp. These features align with previously documented descriptions, confirming the consistency in fruit morphology. Microscopic analysis further supported the identification of ajwain by detailing the anatomical structure of the mericarps, the presence of vittae, epicarp with tangentially elongated cells, and well-differentiated vascular bundles. The presence of schizogenous ducts

and oil globules in the endosperm indicates its potential as a source of essential oils, which are widely used for medicinal and culinary purposes^{17,18}.

Powder Microscopy

Powder microscopy provided additional confirmation of the plant's identity through characteristic features such as papillae on the epicarp, anomocytic stomata, fibers, annular vessels, and oil globules. The presence of these diagnostic features ensures the authenticity of ajwain when processed into powdered forms for medicinal and commercial use. The slightly bitter and aromatic taste further reinforces its traditional applications as a carminative and digestive aid.

Histochemical Analysis

Histochemical testing highlighted the presence of various bioactive compounds contributing to *Trachyspermum ammi*'s pharmacological properties. The detection of cutin in the epicarp cells, phenolic compounds in the endocarp and vittae, and alkaloids in different tissue regions suggests potential antioxidant and antimicrobial activities. The mucilage in the outer layer of the endosperm and papillose outgrowths of the epicarp could contribute to the plant's demulcent and soothing properties. Lignin in the xylem walls indicates structural rigidity, while the presence of oil globules in the endosperm cells supports its traditional use in digestive and respiratory ailments.

Preliminary phytochemical screening

The preliminary phytochemical screening of *Trachyspermum ammi* extracts using various solvents—petroleum ether, chloroform, ethanol, and methanol—revealed the presence of several bioactive compounds, with variation depending on the polarity of the solvent used. These results suggest that the nature and polarity of the solvent play a significant role in the extraction efficiency of phytoconstituents.

Trachyspermum ammi revealed that methanol and ethanol extracts contained the highest number of bioactive compounds. These included alkaloids, flavonoids, tannins, proteins, saponins, and carbohydrates, indicating strong polarity-dependent extraction. Chloroform exhibited minimal activity, whereas petroleum ether revealed the presence of only steroids. The results highlight the predominance of polar phytoconstituents in *Trachyspermum ammi*. This supports its traditional medicinal use and suggests potential for further pharmacological studies.

Physicochemical parameters

The physicochemical evaluation of *Trachyspermum ammi* powder revealed that all parameters, including LOD, ash values, and pH, were within the limits prescribed by the British and Ayurvedic Pharmacopoeias, indicating good quality and minimal contamination. The low moisture content suggests reduced microbial risk and enhanced shelf life. Ash values confirmed the absence of excessive inorganic impurities. The pH was slightly acidic, suitable for herbal formulations. Notably, high water- and alcohol-soluble extractive values reflect the presence of significant phytoconstituents, supporting its traditional medicinal uses. These results validate the plant's suitability for therapeutic applications.

Pharmacological and Medicinal Relevance

The findings of this study corroborate the traditional medicinal uses of ajwain. The presence of thymol, a major bioactive compound in ajwain oil, is known for its antimicrobial, antifungal, and carminative properties. Additionally, the confirmed presence of alkaloids, phenolics, and mucilage suggests potential anti-inflammatory and gastroprotective effects, which align with its traditional applications in treating indigestion, flatulence, and respiratory disorders¹⁹.

CONCLUSION

The pharmacognostic and phytochemical evaluation of *Trachyspermum ammi* establishes a reliable basis for its identification, authentication, and quality assurance in herbal formulations. The integration of macroscopic, microscopic, powder, and histochemical analyses ensures comprehensive characterization, while phytochemical screening confirms the presence of key bioactive compounds. These findings support its traditional medicinal applications and highlight its potential in the development of standardized herbal products. Future studies should aim at quantitative analysis, isolation of active constituents, and detailed pharmacological validation to explore its full therapeutic scope.

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