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Baby Chauhan, Gopal Kumar and Mohammed Ali

Department of Pharmacognosy and
Phytochemistry, Faculty of Pharmacy,
Jamia Hamdard, New Delhi, India

Pharmacognostical and Physiochemical Parameters of *Trachyspermum ammi* (L.) Sprague (Fruits)

Baby Chauhan, Gopal Kumar and Mohammed Ali

ABSTRACT

Trachyspermum ammi (L.) Sprague, syn. *Carum copticum* Benth. et Hook., commonly known as ajwain or Bishop's weeds; belongs to the family 'Apiaceae'. its fruits yielded 2% to 4% brownish essential oil, with thymol as the major constituent (35% to 60%). The present study deals with the Pharmacognostical examinations like morphological and histological characters of fruits of *T. ammi* besides physiochemical, fluorescence and phytochemical analysis. The HPTLC fingerprintings of *T. ammi* (fruits) petroleum ether, dichloromethane, chloroform, methanol, ethanol extract developed in solvent system (toluene: ethylacetate; 93:7) and spotted thymol peaks at UV 366 nm. These observations will enable to standardize the botanical identity of the drug in its crude form. Data evolved in this exploration could be used in laying down pharmacopoeial principles for the drug studied, as standardization of herbal medicines is completely essential.

Keywords: *Trachyspermum ammi* (L.) Sprague, thymol, pharmacognostical examinations, standardization.

1. INTRODUCTION

Trachyspermum ammi (L.) Sprague, syn. *Carum copticum* Benth. et Hook., commonly known as ajwain or Bishop's weeds is an erect, aromatic, annual herb with striate stem¹, white flowers and small brownish fruits. It belongs to the family 'Apiaceae'. Ajwain is grown in Iran, Egypt, Afghanistan and India² (largely in Uttar Pradesh, Bihar, Madhya Pradesh, Punjab, Rajasthan, Bengal, Tamil Nadu and Andhra Pradesh). The fruits possess characteristic aromatic odour and pungent taste due to presence an essential oil mainly composed of thymol (50%), α -cadinol, δ -cadinene, β -caryophyllene and carvacrol^{3,4}. They are used as antispasmodic, stimulant, tonic and carminative and to treat gastric discomfort^{5,6}. It inhibits the bacterial resistant microbial pathogens and is useful as a plant based antibiotic.

The present study provides the pharmacognostic evaluation, i.e. anatomical and microscopic characteristics, physico-chemical parameter, preliminary phytochemical screening and high performance thin layer chromatography (HPTLC) fingerprinting profiles for this plant.

2. MATERIALS AND METHODS

2.1 Plant material

The fruits of *T. ammi* were collected from the local market of Khari Baoli, Delhi and identified by Prof. M. P. Sharma Department of Botany, Jamia Hamdard, New Delhi. A sample of plant material was deposited in the herbarium of the Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi with a voucher specimen number PRL/ JH / 11/ 03.

Correspondence

Gopal Kumar

Department of Pharmacognosy and
Phytochemistry, Faculty of Pharmacy,
Jamia Hamdard, New Delhi, India

E mail:

rxgopaljamiahamdard@gmail.com

2.2 Pharmacognostical studies

The dried powder of the plant (70 g) was extracted with water for 72 hrs. It was filtered and dried on water bath. The percentage yield of the aqueous extract was 3 % w/w.

2.2.1 Macroscopical character

The fruits are about 1.5-3.0 mm long and 1.2-2.8 mm wide, ovoid, mainly occur as entire cremocarps with pedicel attached or detached with bifid stylopod and glabrous cremocarps. Dorsal surface convex with five equally distinct, longitudinal primary ridges; at the summit curved stylopodium, commissural surface flat, showing darker and light coloured longitudinal bands, former representing the position of vittae and vascular bundles ; odour aromatic; taste is slightly bitter giving a sensation of warmth to tongue ⁷.

2.2.2 Microscopical characters

Epicarp is composed of polygonal cells. In the mesocarpic region, reticulate and lignified parenchyma are seen at vascular strands. Endocarp consists of narrow elongated cells having a parquetry arrangement. Tracheids show helical thickening. Polyhedral, thick walled endosperm contains aleurone grains and oil globules. Vittae are six in number, four on the dorsal surface at the mesocarpic region below the secondary ridges and two on the commissural surface of the mericarp. Vittae long, slender composed of thin walled polygonal cells and is lined by an epithelium of small polygonal tubular cells; 10-15 separate, septum transverse or curved.



Figure 1.1: *Trachyspermum ammi* (L.) Sprague, fruits

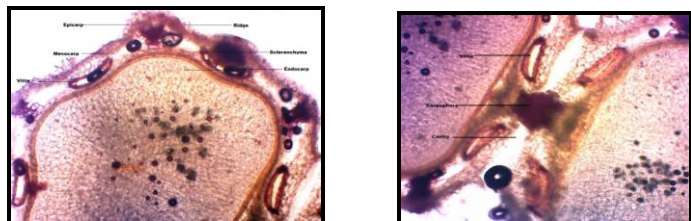
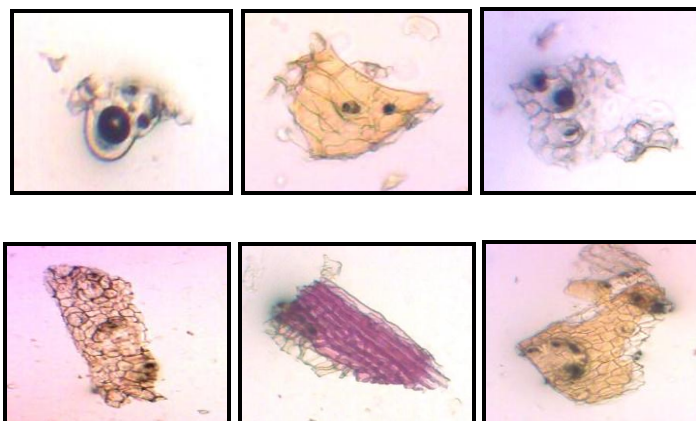


Figure 1.2: T.S. of *T.ammi* fruits



(A) Endocarp in surface view (B) Detached protuberance (C) Microsette crystals (D) Striated cuticle in surface view (E) Pitted fibres (F) Endosperm containing microsette crystal of Ca-oxalate

Figure 1.3: Powder analysis of *T.ammi* fruits

2.3 Preliminary Phytochemical Screening

The phytochemical screening involves testing of different plant extracts for their contents of different classes of compounds^{8, 9, 10}. Preliminary phytochemical screening of the extract for different types of chemical constituents should be followed by qualitative chemical tests to give general idea regarding the nature of chemical constituents present in the crude drugs (table 1.1)^{11, 12}.

2.4 Physicochemical parameters

2.4.1 Fluorescence analysis

Many herbs fluorescence when cut surface or powder is exposed to UV light and this can help in their identification method. Powdered drug (40 mesh) was treated with different reagents and examined under UV light (254 and 366 nm) (Table 1.2).

2.4.2 Determination of ash value

This parameter is used for determination of inorganic materials, e.g., carbonates, silicates, oxalates and phosphates.

Total ash – The ground drug (2 g) was incinerated in a silica crucible at a temperature not exceeding 450⁰ C until free from carbon. It is then cooled and weighed to get the total ash content (Table 1.3).

Acid insoluble ash – The ash was boiled with 25 ml of dilute HCl (6N) for 5 minutes. The insoluble matter collected on ash-less filter paper, washed with hot water and ignited at a temperature not exceeding 450⁰C to a constant weight (Table 1.3).

Water soluble ash – The ash was dissolved in distilled water, the insoluble part collected on an ash-less filter paper and ignited at 450°C to a constant weight (Table 1.3).

Table-1.1. Preliminary phytochemical screening of methanolic extract *T. ammi* (fruits)

S. No.	Constituents	<i>T. ammi</i>
1	Alkaloids	–
2	Carbohydrates	–
3	Glycosides	+
4	Tannins	–
5	Flavonoids	+
6	Proteins and free amino acids	–
7	Saponins	–
8	Mucilage	–
9	Resins	–
10	Lipids / fats.	+

+ Present, - Absent

2.4.3 Determination of extractive values

These values provide an indication of the extent extractive values and are determined according to the method described in pharmacopoeia.

Individual extractives - The air-dried coarse drug powder (5 g) was macerated with solvent (100ml) in a closed flask for 24 hours, shaking frequently during every six hours and allowing to stand for 24 hours. It was filtered rapidly, taking precaution against loss of the solvent. The filtrate was evaporated to dryness in a tared flat bottom dish, dried at 105°C, to constant weight and weighed (Table 1.4).

Successive soxhlet extractives - The powdered material of the drug (5 g) was packed in a Soxhlet apparatus and subjected to successive extraction with different solvents like petroleum ether, benzene, chloroform, methanol and water. The extracts were evaporated to dryness and their constant extractive values were recorded (Table 1.4).

Table 1.2: Fluorescence behaviour of powdered drug of *T. ammi*

S.No.	Chemical treatment	Day light	At 254 nm	At 366 nm
1	Powder as such	Light brown	Brown	Fluorescent yellow
2	1N NaOH in water	Yellowish brown	Greyish yellow	Yellow
3	1N NaOH in methanol	Yellowish brown	Brown	Pale green
4	Iodine in water	Black	Brown	Fluorescent blue
5	1 N HCl	Brown	Greyish yellow	Brown
6	Conc. HNO ₃	Reddish brown	Yellow	Fluorescent blue
7	Conc. H ₂ SO ₄	Brown	Greyish yellow	Light green
8	50% KOH	Yellowish brown	Greyish yellow	Fluorescent blue
9	50% H ₂ SO ₄	Brown	Yellow	Light green
10	50% HNO ₃	Reddish brown	Yellow	Fluorescent blue

Table-1.3: Ash values of *T. ammi*

S.No	Ash Values	Values (% w/w) mean (n=3) ±SD
1	Total ash	8.5±0.006
2	Water soluble ash	7.7±0.01
3	Acid insoluble ash	0.17±0.01

2.4.4 Loss on drying

This parameter determines the amount of moisture as well as the volatile components present. The powdered drug sample (10 g) was placed on a tared evaporating dish, dried at 105°C for 6 hours and weighed. The drying was continued until two successive reading matches each other or the difference between two successive weighing was not more than 0.25% of constant weight. The loss of drying (LOD) was found to be 4.5±0.036 % w/w [mean (n=3) ±SD].

Table-1.4: Individual and successive extractive values of *T. ammi* fruits

Individual extractive value	Values (% w/w) mean(n=3)±SD	Successive extractive value	Values (% w/w) mean(n=3)±SD
Petroleum Ether extract	5.4±0.03	Petroleum Ether extract	5.4±0.02
Chloroform extract	5 ±0.01	Chloroform extract	2.8±0.01
Methanol extract	6.2±0.02	Methanol extract	7.6±0.006
Acetone extract	4.4±0.01	Acetone extract	3.6±0.02
Hydroalcoholic extract	6.6± 0.01	Hydroalcoholic extract	12 ±0.04
Water extract	15.2±0.03	Water extract	19.4±0.05

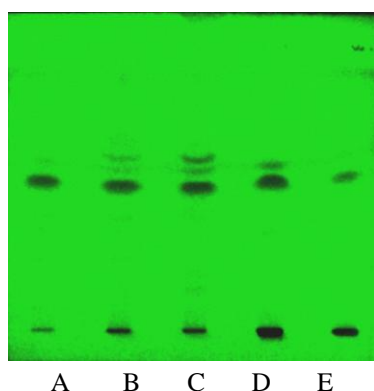
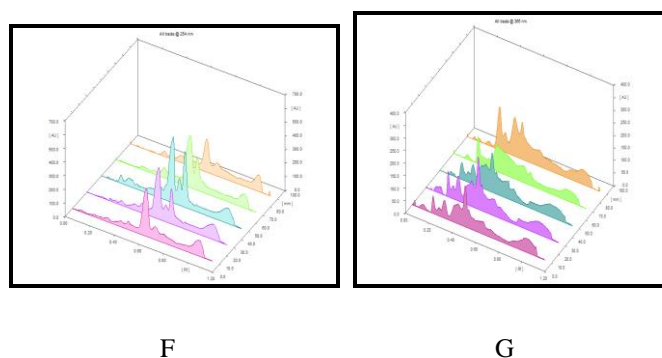


Figure-1.4: HPTLC fingerprinting of Petroleum ether extract (A); Dichloromethane (B); Chloroform extract (C); Methanol extract (D); Ethanol extract (E) at UV 366 nm

Figure-1.5: 3-D display of the chromatogram of methanolic extract of *T. ammi* (fruits) at UV 254 nm (F) and 366 nm (G).

2.5 HPTLC fingerprinting

2.5.1 Preparation of plant material

The powdered plant material (15 g) was extracted individually with 100 ml each of methanol, Petroleum ether, Dichloromethane, Chloroform, Ethanol by sonicator for 30 minute and filtered. Each extract was concentrated under reduced pressure separately.

2.5.2 TLC Fingerprinting

Sample spots were separated on TLC plates using solvent system of toluene: ethyl acetate (93:7) as developing solution¹³. Various visualisation techniques were used to obtain best TLC fingerprint, like UV radiation at 254 nm, UV 366 nm, iodination and spray reagents, e.g., anisaldehyde, vanillin and sulphuric acid^{14, 15}.

2.5.3 HPTLC Scanning

The developed plate were scanned under the Camag HPTLC scanner IV for the densitometric observation. The plate was scanned with UV 254 and 366 nm using Toulene:Ethyl acetate (19:1) as a solvent system.

3. RESULT AND DISCUSSION

In the present study the pharmacognostic evaluation, i.e., anatomical and microscopic characteristics, physic-chemical parameters, preliminary phytochemical screening and high performance thin layer chromatography (HPTLC) fingerprinting profiles were performed.

A transverse section of the fruits showed the presence of polygonal cells, reticulate and lignified parenchyma, tracheids, aleurone grains, oil globules and vittae (Figure 1.2).

The powder microscopy of the *T. ammi* showed the presence of thin walled parenchymatous cells in groups; pitted fibers and endosperm containing microrosette crystal of Ca-oxalate. The measurements of various cell and tissues are provided respectively (Figure 1.3).

Preliminary phytochemical test of the methanol extract of the drug indicated the existence of glycosides, flavonoids and terpenoids (Table 1.1).

The fruit powder was studied for its physico-chemical constants which included ash values, fluorescence analysis, individual and successive extractive values and LOD. It exhibited

higher hydroalcoholic and water extractive contents (Table 1.2, 1.3 and 1.4).

HPTLC fingerprinting was performed for the different extracts, e.g., petroleum ether, dichloromethane, chloroform, methanol and ethanol of *T. ammi* (fruits). The best solvent system for *T. ammi* (fruits) extracts was found to be toluene: ethyl acetate (93:7) which showed better separation and high resolution.

The HPTLC fingerprintings of *T. ammi* (fruits) petroleum ether, dichloromethane, chloroform, methanol and ethanol extracts developed in the solvent system (toluene: ethylacetate; 93:7) were scanned at two wavelengths UV 254 nm and UV 366 nm. At UV 254 nm, 9 peaks in the petroleum ether; 10 peaks in dichloromethane, 11 peaks in chloroform, 6 peaks in methanol, 7 peaks in ethanol were observed in chromatogram. Major peaks with high intensity of compounds were observed at R_f values 0.91, 0.74, 0.63, 0.59 in petroleum ether extract; 0.81, 0.7, 0.63, 0.54 in dichloromethane, 0.78, 0.70, 0.63, 0.54 in chloroform; 0.68, 0.59, 0.39, 0.31 in methanol extract; 0.71, 0.60, 0.48, 0.42 in ethanol extract.

4. CONCLUSION

The pharmacognostic study of the fruits of *Trachyspermum ammi* (L.) Sprague has shown certain microscopic feature and preliminary phytochemical data of diagnostic values. Physico-chemical constants such as solubility, ash values, individual or successive extractive values and other parameters including fluorescence analysis and preliminary phyto-chemical studies are important parameters for standardizations of the drug.

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