



E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2018; 6(6): 122-126
Received: 21-09-2018
Accepted: 25-10-2018

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Pharmacognostical studies on the fruits of Vanya Ajamoda *Trachyspermum roxburghianum* (Dc.) H. Wolff

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Abstract

'Ajamoda' refers to an important drug used in Ayurvedic system of medicine, which consists of umbelliferous fruits. As per Ayurvedic Formulary of India, part-3, the botanical equivalent for Ajamoda is *Apium leptophyllum* (Pers.) Muell., but in market samples, the fruits of *Trachyspermum roxburghianum* (DC.) H. Wolff and *Apium graveolens* are commonly used substitutes. In order to identify the each drug individually from each other of these 3 fruits, first we have attempted to establish the pharmacognostical standards for *Trachyspermum roxburghianum* (DC.) H. Wolff as per the latest Ayurvedic Pharmacopoeia of India (part-1, volume-8). The microscopical studies on the fruits revealed the presence of prominent hispid cremocarps with five distinct longitudinal ridges in each mericarp with warty surface, commissural surface flat, odour strongly aromatic. Microscopical studies showed the presence of papillose epidermis, warty unicellular rounded and T shaped trichomes, collateral vascular bundles, oil globules and microrosette crystals of calcium oxalate in endosperm region. Physicochemical analysis for powder drug, water extract and water-alcoholic extract has been carried out in order to establish the pharmacognostical standards for the fruits of *T. roxburghianum* (DC.) H. Wolff.

Keywords: Ajamoda, vanya ajamoda, trachyspermum roxburghianum, pharmacognosy, physico-chemical

1. Introduction

In Ayurvedic system of medicine 'Ajamoda' is a well known reputed drug, which is used in the single name with different botanical sources like *Apium leptophyllum* (Pers.) Muell., *Apium graveolens* and *Trachyspermum roxburghianum* (DC.) H. Wolff. As per Ayurvedic Formulary of India volume 1, 2 and 3, Ajamoda is used in more than 35 formulations i.e. abhadyaurna, abhrakadi vati, agnikumara rasa, agnitunda rasa, agnitundi vati, ajamodadiurna, ajamodarka, bhasma vati, brhat haridrakhanda, brhat saindhavadhya taila, citrakadiurna, dadhika ghrita, kalyanavaleha, kankayana gutika, krmimudgara rasa, mahasarasvathiurna, samudrayaurna, sarasvataurna, vaisvanaraurna, vanya ajamodarka, vidalavanadi guti, vrddhihari vatika etc. to mention a few. As per Ayurvedic Formulary of India, the accepted source for Ajamoda is *Apium leptophyllum* (Pers.) Muell., and the dosage mentioned is 1-3 gm of the drug in powder form and 10-20 ml twice a day in arka form [1, 2, 3].

Trachyspermum roxburghianum (DC.) H. Wolff belonging to the family Apiaceae is a much branched, annual herb, grows up to 90 cms in height, cultivated all most throughout India and grows as weed also. Leaves bipinnately divided, ultimate segments of the lower leaves rather broad, of the upper narrowly linear-lanceolate. Flowers are small in terminal or axillary, compound umbel, white or greenish white in colour. The fruits constitute one of the lesser-known spices used for flavouring curries, either alone or in mixture with other species and condiments. They are also used in the preparation of pickles, chutneys and preservatives. The leaves are used as a substitute for parsley [4].

The drug occurs mostly as entire cremocarps, with or without the pedicle and a bifid stylopod. A small proportion may occur as separate mericarps, which are broadly ovoid, more or less curved, and possess five distinct longitudinal ridges. The drug has an aromatic odour and the taste is at first slightly bitter becoming strongly aromatic and producing a slight numbness of the tongue. The fruits form an ingredient of carminative and stimulant preparations and are useful in dyspepsia. It is also much used as a cardio tonic, emmenagogue and in bronchitis and asthma [5, 6]. Ajamoda kshreera swedana, when administered internally for children is useful for Ajirna, Agni mandya and kostakrimi [7]. The fruits yield a greenish yellow essential oil by steam distillation up to 2.5% and 4.5% fixed oil.

Regional language names: Sanskrit: vanya ajamoda, ajamoda, ayamoda, ajamoja; English: ajowan; Hindi: ajmud, radhuni, randhuni; Kannada: ajamodhavoma;

Malayalam: ayamodakam; Tamil: asamtavomam, ashamtagam; Telugu: ajumoda, vamu [5].

Synonyms: *Trachyspermum involucreatum* (DC.) H. Wolff; *Trachyspermum stictocarpum* (C.B. Clarke) H. Wolff; *Apium involucreatum* Roxb.; *Ptychotis roxburghiana* DC. Lindl.; *Pimpinella involucreata* (Roxb.) Wight & Arn [8].

In order to overcome the confusion because of morphological similarity and controversy among umbelliferous fruits as Ajamoda sources, the present study has been taken up to study the important identification characteristics and to establish pharmacopoeial standards of *Trachyspermum roxburghianum* (DC.) H. Wolff, which is also using as ajamoda, as per latest Ayurvedic Pharmacopoeia of India (part-1, volume-8).

2. Materials and Methods

The dried fruits were procured from crude drug sellers from Maharashtra state, and authenticated by survey of medicinal plants unit, Regional Ayurveda Research Institute for Metabolic Disorders, Ashoka pillar, Jayanagar, Bengaluru.

The microscopical characters of the fruits were noted. For powder microscopy study, the powder was stained with phloroglucinol and concentrated HCl to study the lignified cells, trichomes, fibres, xylem vessels, etc. The powder was also stained with N/50 iodine solution to detect the presence of starch. A small portion of powder was mounted in water to identify calcium oxalate crystals. Microscopy of the fruits was carried out by the methods prescribed by Trease and Evans [9, 10]. Camera lucida drawings were drawn with the help of mirror type camera lucida.

Water and hydro-alcoholic (50:50) extractive values were determined according to the standard and latest Ayurvedic Pharmacopoeial methods by using water bath. For this purpose the powder (100g) was extracted 3 times (each 300 ml) with water and water-alcohol each for 16 hours. The dried extractives were obtained after evaporation of solvent under reduced pressure by rotary evaporator. Physico-chemical parameters such as ash values, alcohol soluble and water soluble extractive values and loss on drying, pH, total dissolved solids for extracts of flowers were determined as per the standard Ayurvedic Pharmacopoeial methods and recorded in Table-2 [10, 11].

3. Results

3.1 Macroscopic characteristics

Fruit, occurs mostly as entire cremocarps with pedicel attached or detached at the base and bifid stylopod at the apex, broadly ovoid 1.5 cms to 3 mm in length and 1.2 to 2.8mm in width, light brown, dorsal surface convex with five distinct longitudinal ridges in each mericarp, surface warty, commissural surface flat, showing two darker longitudinal bands Representing the vittae. Odour, strongly aromatic, taste slightly pungent (Figure-1).



Fig 1: Dried fruits

3.2 Microscopic characteristics

Diagrammatically fruit shows 5 strongly well developed primary ridges each with a vascular bundle, 4 large vittae on the dorsal surface and 2 on the commissural surface, where lies raphe in between the endocarp and testa layer. Diagrammatic L.s of the fruit shows cylindrical embryo embedded in the endosperm, towards the apical portion of the fruit. Epicarp consists of highly papillose epidermis, interrupted at places with warty unicellular trichomes, with striated cuticle, mesocarp parenchymatous, vascular bundle collateral, with xylem and phloem, vittae broad, lined with dark brown epithelium layer, endocarp composed of unequal sized tangentially running thin walled parenchymatous cells. Testa layer very broad, cells of the endosperm thick walled, parenchymatous and filled with oilglobules and aleurone grains with microrosette crystals of calcium oxalate (Figure-2).

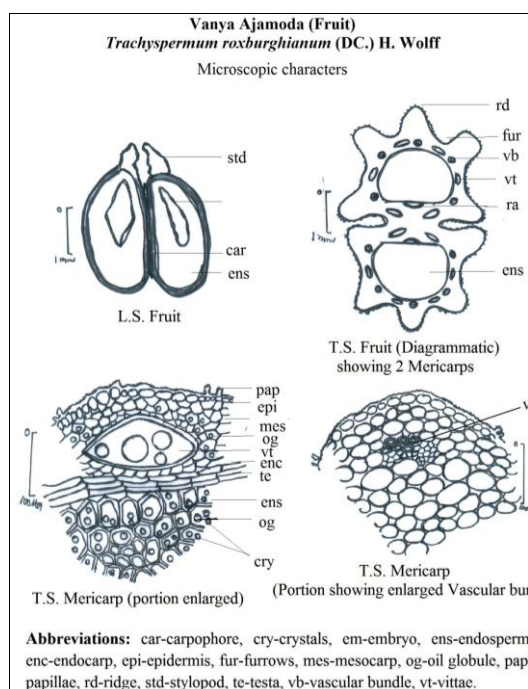


Fig 2: Fruit microscopy

3.3 Powder microscopy

Powder light brown in colour with strong pleasant odour, shows abundant warty unicellular trichomes, with few rounded trichomes, and 'T' shaped trichomes, epidermal cells in surface view, thick walled groups of endosperm cells, which are unligified, with abundant oil globules and

microsette crystals of calcium oxalate, parenchyma cells with xylem parenchyma, debris of xylem vessels with helical type, fragments of trichomes with epidermal cells, groups of thin walled mesocarp cells and thin walled parenchymatous cells and fragments of testa cells (Figure-3).

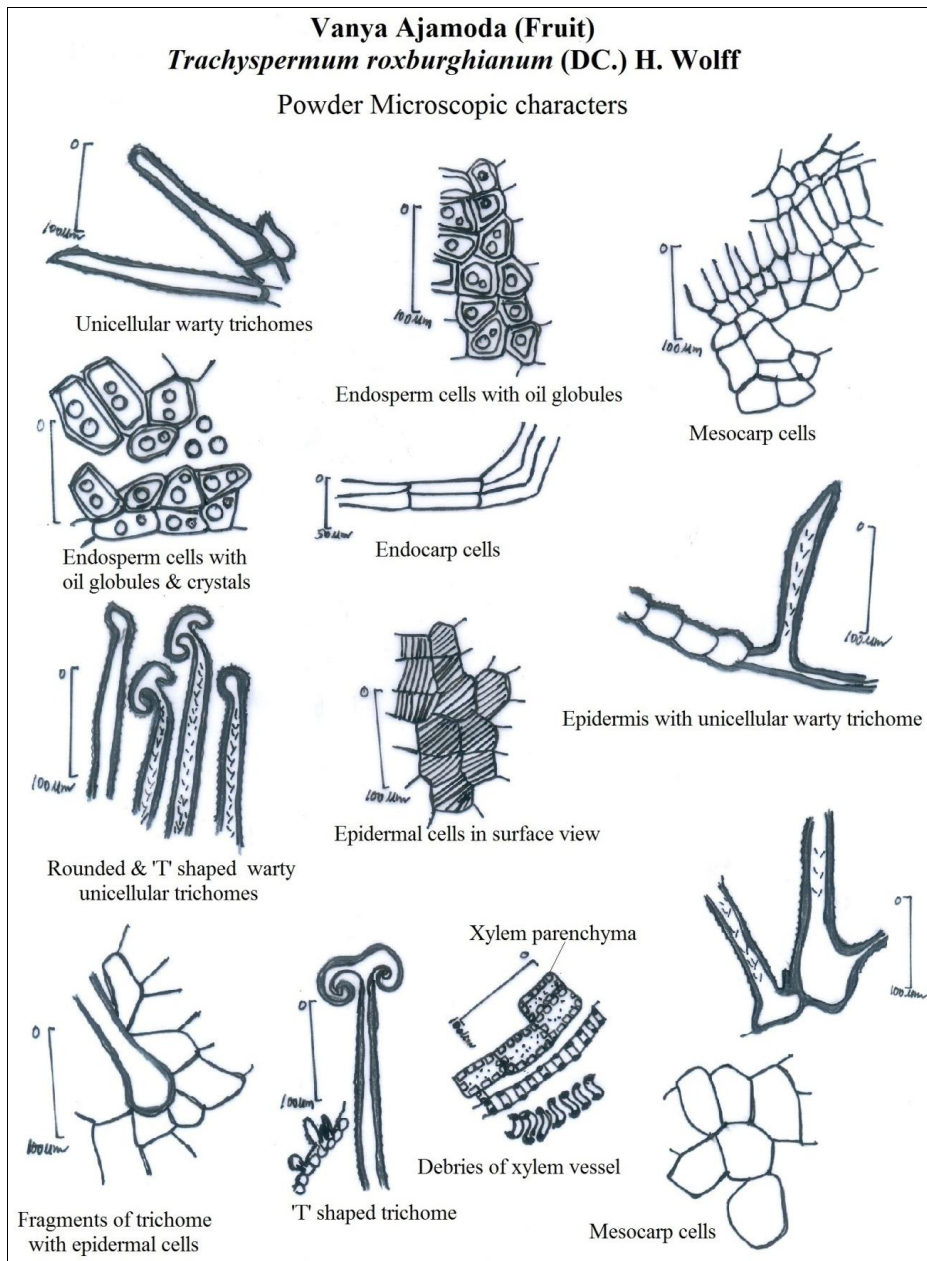


Fig. 3: Powder microscopy

3.4 Physico-chemical analysis

Physicochemical analysis such as total ash, acid-insoluble

ash, loss on drying at 105⁰ C, have been carried out and the results were given in table 1.

Table 1: Physicochemical parameters *T. roxburghianum* – Fruits powder

S. No.	Name of the parameter	Values (%) w/w
1.	Foreign matter	2.0%
2.	Loss on drying at 105 ⁰ C	14.16
3.	Total ash	10.78
4.	Acid-insoluble ash	4.13
5.	Water-soluble extractive	15.64
6.	Alcohol-soluble extractive	7.39
7.	Water-Alcohol (50:50) soluble extractive	19.5

3.5 Hhydro-ahlcoholic extract: 100g of suitably sized powder was taken in an extractor and added 50 per cent

aqueous alcohol, about 3 times the quantity of raw material and refluxed at a temperature between 80-85⁰C for 3-4 hours.

Filtered the extract through a Whatmann no.1 filter paper to a suitable vessel. The marc was extracted three times more, filtering the extract each time into the same vessel. The combined filtrates were concentrated to syrupy consistency and dried by using a rotary evaporator at a temperature not exceeding 80°C. The yield obtained is about 19.5 per cent. The physicochemical analysis for dried hydro-alcoholic extract have been carried out and results showed in table-2.

3.6 Water extract: 100g of suitably sized powder was taken

in an extractor and added with thrice the quantity of distilled water, and heated at 80-85°C for 3-4 hours. The extract is filtered through a Whatmann no.1 filter paper to a vessel. The marc was extracted three times more, filtering the extract each time into the same vessel. The combined filtrates were concentrated to syrupy consistency and dried by using a rotary evaporator at a temperature not exceeding 80°C. The yield obtained is about 15.78 Per cent. The physicochemical analysis for dried water extract has been carried out and results showed in table-2.

Table 2: Physicochemical parameters of *T. roxburghianum* – Fruit extracts

S. No.	Name of the parameter	Values (%) w/w	
		hydro-alcoholic extract	water extract
1.	pH (5 % w/v aq. solution)	6.05	6.95
2.	Loss on drying at 105°C	3.35	1.21
3.	Total ash	16.80	11.10
4.	Acid-insoluble ash	0.17	0.15
5.	Total soluble solids	95.39	96.64

3.7 Thin Layer Chromatography

2.0 g of the powdered drug material was taken with 25 ml of methanol and heated on a water bath for 10 minutes, cooled and filtered. The hydro-alcoholic and water extract test solutions were prepared by dissolving 10 mg of the dried extracts each in 10 ml of methanol and filtered. The filtered test solutions were applied on a precoated silica gel 60₂₅₄ TLC plate as bands. The plates were developed to a distance of 8 cm from the line of application by using *Toluene: ethyl acetate* (9:1) as mobile phase. The plates were then dried in

air and examined under 254 nm. After air drying plates were sprayed with *Anisaldehyde sulphuric acid reagent*. Heated the plate at 110°C for about 5 minutes and measured the R_f values of different extracts. The chromatograms obtained after spraying with *Anisaldehyde sulphuric acid reagent*, powdered drug showed bands at R_f 0.05, 0.15, 0.20, 0.27, 0.34, 0.38, 0.42, 0.47, 0.55, 0.63, 0.69, 0.81, 0.85; hydro-alcoholic extract at R_f~ 0.05, 0.15, 0.20, 0.27, 0.34, 0.38, 0.42, 0.52, 0.55, 0.63, 0.74, 0.81 and water extract at R_f~ 0.05, 0.15 (Figure-4).

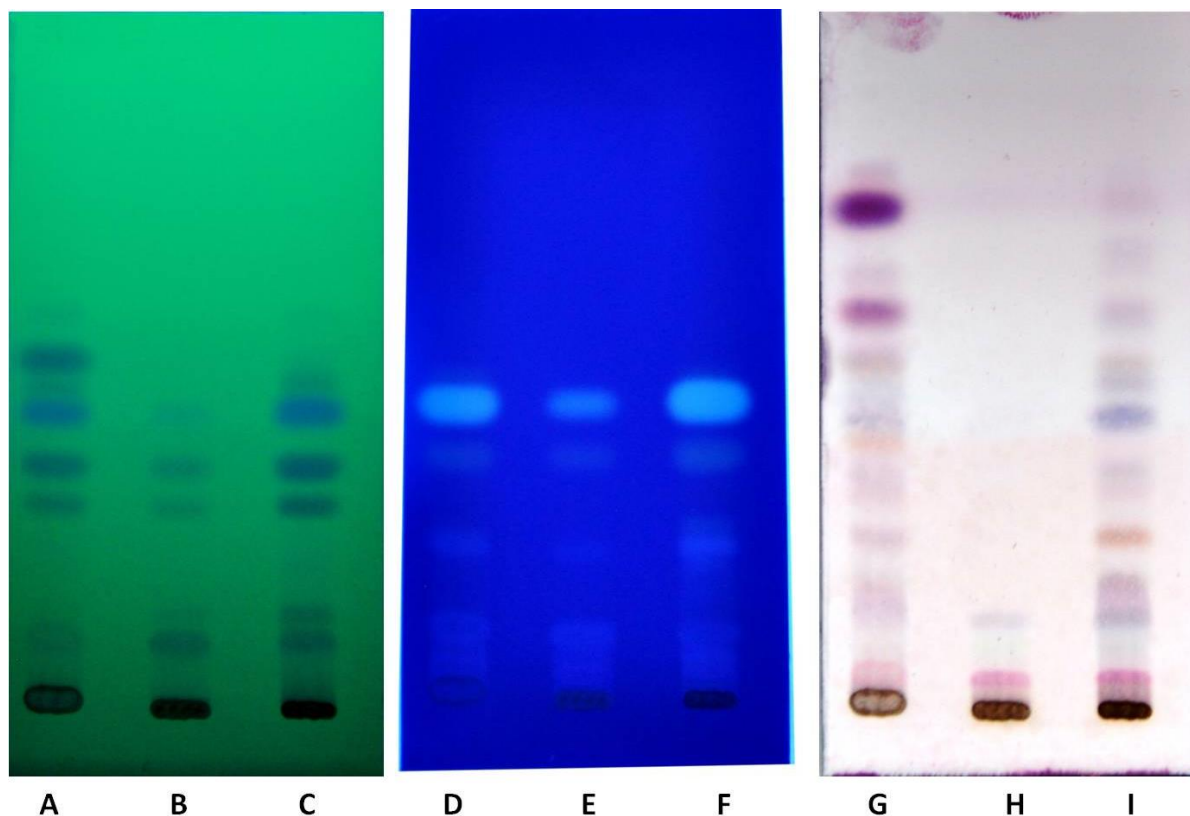


Fig. 4:Thin Layer Chromatography: A-Powder, B-Water, C-Wat-Alc. Ext. under 254 nm; D-Powder, E-Water, F-Wat-Alc. Ext. under 356 nm ; G-Powder, H-Water, I-Wat-Alc. Ext. after spraying with *Anisaldehyde sulphuric acid reagent*.

4. Conclusion

In terms of Pharmacognosy, the following features are striking to the fruits of vanya ajamoda - *Trachyspermum roxburghianum* (DC.) H. Wolff and can serve as principal parameters for authentication in fresh as well as dry form (including powder form). Macroscopically fruits of *T. Roxburghianum* shows prominent ovoid-oblong hispid (hairy) cremocarps with five distinct yellowish longitudinal ridges, surface warty and light brown in colour, measures 3.0 to 4.0 mm long and 2.0 to 2.5 mm broad. Microscopically fruits exhibits important characters like papillose epidermis, warty unicellular rounded trichomes, 'T' shaped curved trichomes, collateral vascular bundles, oil globules and microrosette crystals of calcium oxalate in endosperm region. Microscopically powder shows presence of abundant warty unicellular trichomes, few rounded trichomes, abundant oil globules, xylem parenchyma, debris of xylem vessels with helical type.

Based on these macro, microscopical characteristics and physico-chemical data evolved from the present investigation may be utilized for the identification and standardization of the drug in order to check and ensure the quality of the drug in quality control laboratories, to differentiate from other similar looking drugs i.e. *Apium leptophyllum* (Pers.) Muell., & *Apium graveolens* and for laying down Pharmacopoeial standards for the fruits of vanya ajamoda - *Trachyspermum roxburghianum* (DC.) H. Wolff.

5. Acknowledgement

Authors are thankful to the Director General, CCRAS, New Delhi, for providing necessary facilities and encouragement to carry out the work successfully.

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