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Identification of the Botanical Sources and TLC/HPTLC Pattern of 'Ajamoda' In Trade

SaraswathyA^{1*}, Amala K¹ and Vidhya B¹

1. Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS) Arignar Anna Hospital Campus, Arumbakkam, Chennai – 106, India.
[E-mail: csmdria@gmail.com, saraswathy20002004@gmail.com, Ph: 044- 2621 4823, Fax: 044- 2620 7566]

'Ajamoda' is an important drug of the Ayurveda, Siddha and Unani systems of medicine, which consists of umbelliferous fruits. The drug to be used in the formulations are fruits of *Apium leptophyllum*. Confusion exists in literature regarding its botanical identity. Ajamoda and similar names have been very freely used for several related umbelliferous fruits viz. *Apium leptophyllum*, *Apium graveolens*, *Trachispermum roxburgianum*. However, an examination of the various market samples of ajamoda revealed the presence of only three of the umbelliferous fruits viz. *Apium leptophyllum*, *Apium graveolens*, *Trachyspermum roxburgianum*. The present study revealed the botanical identity and TLC/HPTLC aspects of three ajamoda fruits, which will be useful in identification of the particular drug.

Keyword: *Ajamoda*, Umbelliferae, Botany, TLC, Fingerprinting.

1. Introduction

'Ajamoda' is an important Ayurvedic plant drug appearing in several compound formulations admitted to the Ayurvedic Formulary of India, Parts I and II. It is frequently used for the relief of a number of ailments viz. bronchitis, asthma, hiccough, carminative, stimulant, cardial and pain in the bladder^[1-8]. It is now generally believed that 'Ajamoda', which consists of umbelliferous fruits, is not available in pure form from one botanical source, but only as a mixture of two or three different umbelliferous fruits viz. *Apium leptophyllum* (Pers.) F. Muell ex Benth, *Apium graveolens* Linn. and *Trachyspermum roxburgianum* (DC) Craib. The AFI mentions that the botanical source of Ajamoda is *Apium leptophyllum*. According to botanical literature on medicinal plants, these are either under

cultivation, or growing in wild, in the northern hills and plains from the lower hills of J & K State to Eastern Uttar Pradesh. Therefore, the present paper attempts to reveal the botanical identity and TLC/HPTLC finger print profile of three umbelliferous fruits for identification of the drug.

2. Materials and methods

The dried samples were obtained from drug dealers in different market and identified with the help of reference samples and the same was procured from New Delhi and Jammu. Identification of the collected samples was done by studying their macro and microscopic and comparing them with the characters mentioned in various texts and published papers.

The reference samples No. (*A.l*-Fr5, *A.g*-Fr5a, *T.r*- Fr21a) deposited in the crude drug museum of the Captain Srinivasa Murti Drug Research Institute in Ayurveda and Siddha, Chennai.

The samples were examined under stereomicroscope, at 20 to 40x. Mixtures were separated by handpicking. Separated fruits were

swirled and washed in water to remove particles adhering due to cross contamination. A portion was then treated with chloral hydrate solution, washed thoroughly in water, and stored in alcohol-glycerol mixture. Another portion was heated with 2.5 per cent KOH, washed in water and stored similarly.

Table 1: Diagnostic Macroscopic characters of three umbelliferous fruits

Features	<i>Apium leptophyllum</i> (Pers.) F. Muell ex Benth.	<i>Apium graveolens</i> Linn.	<i>Trachyspermum roxburghianum</i> (DC.) Craib
Creomocarp (Macromorphology)			
Colour	Light brown to Yellow	Brownish yellow / creamy	Grayish
Shape	Ovoid to spherical with a basal sinus; separated mericarps not curved;	Oblong to conical, laterally compressed, with a rounded base; separated mericarps not curved;	Conical with rounded base; separated mericarps curved;
Size	2mm or over in length and about 2mm. in width at the middle; largest of the three;	About 1.5 mm long, and about 1mm wide at the middle; smallest of the three;	About 2mm. long and 1.5 mm wide at the middle
Surface	Glabrous to naked eye, as well as at 10 to 20x; ridges straight, yellow and valleculeae, broad and reddish brown;	Glabrous to naked eye, but rough surface and minute projections at 10 to 20x; valleculeae narrow and dark; ridges often wavy;	Ridges straight, grayish; valleculeae narrow and almost blackish; hispid, hairs visible to naked eye;

Fruits were removed from storage, kept in a small watch glass in water, and examined under 20 to 40x under stereomicroscope. Tissues were separated with needle, epidermal peel teased out and mounted to get both the top and reverse surface views. Similarly, tissues from the mesocarp, major and minor vittae, endocarp, vascular tissues were all tested out and mounted in pure glycerin for microscopy. Seed were removed intact, crushed and mounted. Slides were prepared with tissues from both chloral hydrate and KOH treated materials. Measurements were made with micrometer and drawings made with the help of camera lucida.

The coarsely powdered drug (2 g) was extracted separately with 100 ml of *n*-hexane and ethyl acetate using Soxhlet apparatus. Filtered and concentrated the combined extract under vacuum and made up the volume to 10 ml with respective solvents and used this solution for TLC. Linomat IV (CAMAG, Switzerland) applicator was used for sample application. Applied 10 µl of *n*-hexane and ethyl acetate extracts separately in the mobile phase of Toluene: Ethyl acetate (7:1) and Toluene: Ethyl acetate (4:1) respectively, the plates were developed up to the height of 8 cm from the point of application. The developed plates were air dried and viewed under UV chamber at 254 nm and 366 nm and the images were recorded. The plates were densitometrically

Table 2: Microscopic characters of three umbelliferous fruits (Figure 1-3)

Features	<i>Apium leptophyllum</i> (Pers.) F. Muell ex Benth.	<i>Apium graveolens</i> Linn.	<i>Trachyspermum roxburghianum</i> (DC.) Craib
Mericaip (Micromorphology)			
Epicarp	Epidermal cells mostly straight walled; upto 80 microns long, or occasionally longer, and upto 50 microns broad generally; papillae present, more obvious at the edges;	Epidermal cells upto 50 microns long and 30 microns wide, wavy walled particularly in the vallecular region; papillae present, occasionally rising as fingerlike processes upto about 30 microns, and warty; a few longer ones more seen from the valleculae region;	Epidermal cells straight walled, not exceeding about 70 microns in length, and not exceeding 45 microns in width in general; characteristic trichomes present even upto 500 microns mostly with a head, and several shorter ones; all warty;
Mesocarp	Patchy extensions of the oblong pitted sclerenchymatous cells of the vascular system laterally into the mesocarp are present, but a complete mesocarpic sclerenchymatous layer is absent;	No sclerenchymatous cells except the ones present at the vascular region;	No sclerenchymatous cells except the ones present at the vascular region;
Endocarp	No parquetry arrangement	Parquetry arrangement present sporadically over small areas.	No parquetry arrangement
Vittae	Length upto 1500 microns, and midwidth upto 215 microns; does not extend into the stylopod tissues; end cells bluntly conical;	Length upto 1200 microns, and midwidth upto 135 microns rarely extending into the stylopod region; end cells bluntly conical;	Length upto even 3mm, curved and prolonged into thread like ends, extending into the stylopod region; width upto 250 microns; apart from main and subsidiary vittae, tiny ones like off-shoots from subsidiary vittae present.
Vascular bundles	Each ridge is supported by a vascular bundle, with tracheids, spiral and reticulate vessels and sclerenchyma cells showing oblong pits; narrow xylem fibres are also present.		
Seed	Endospermous, convex on dorsal side and more or less flat on the commissural side; testa a single layer on the dorsal side, but with some more tissue layers on the commissural side; cells of the testa oblong to rectangular, thin walled; embryo embedded in the endosperm; cell walls of the endosperm uniformly thick, cells packed with aluerone grains; many aluerone grains indicate the presence of a tiny speck of calcium oxalate crystal, revealed only under polarizerd light.		

scanned with a CAMAG TLC scanner 3 equipped with win CATS V 4.6 software for finger print profile study at UV λ_{max} 254 nm using deuterium light source. The plates were then derivatized with vanillin-sulphuric acid reagent and on subsequent heating at 105°C till the appearance of the colour of the bands^[9-11].

3. Result and discussion

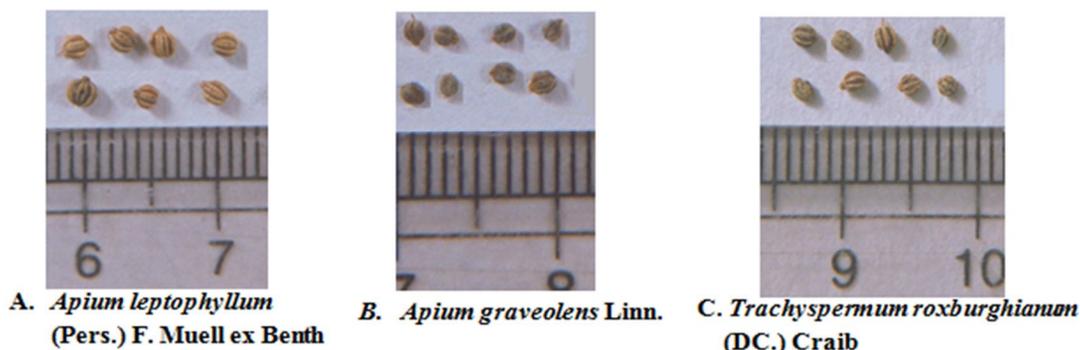
3.1 *Apium leptophyllum* (Pers.) F. Muell ex Benth.

It is an annual herb cultivated in Andhra Pradesh, Gujarat, Madhya Pradesh and Karnataka; Fruits, more cremocarps than mericarps: the cremocarps are ovoid to spherical, showing a sinus at the stalk end, and topped by a bifid stylopod if intact,

but otherwise tops are conically crowned; colour light brown to yellow, glabrous (Plate I-A); cremocarps show a smooth outline, and glabrous surface, under transmitted light (Plate II-A); mericarps are dorsally convex and commissurally

flat surfaced; examined by naked eye or at about 10 to 20x, there are five ridges to each mericarp, three dorsal ones that are longer than the two lateral ones, and curve at the stalk end.

Plate-1
Ajamoda – Comparative sizes of fruits



3.2 *Apium graveolens* Linn.

It is a biennial herb. *A. graveolens* is the source of celery and is cultivated as a cold weather crop in India. In Bengal it is cultivated for its fruits which are used as a spice. Fruits, more mericarps than cremocarps; cremocarps slightly conical, rounded base, not sinusoid (Plate I-B); fruits grayish brown; ridges present, not very prominent, cream coloured many of which are

wavy, vallecule narrow, blackish, occasionally showing minute projections visible at 20 x; ridges are five to each mericarp, all of the same length; stylopod mostly conical, occasionally divided in intact cremocarps; mericarp convex on the dorsal and almost flat on the commissural surface; whole cremocarps seen in transmitted light at 10 to 20x, reveal a rough outline, with minute projections (Plate II-B).

Plate-2 (x 12)
Comparative Macro- and Micro morphology of the three fruits of 'Ajamoda' In Transmitted Light

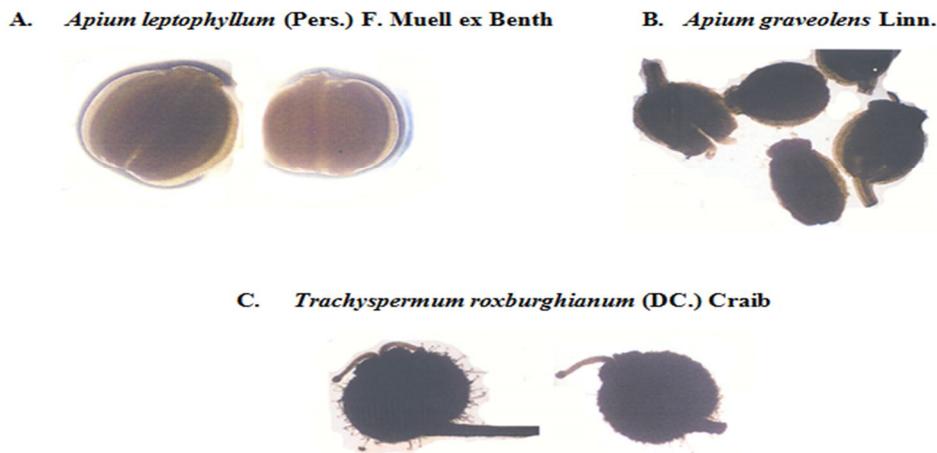
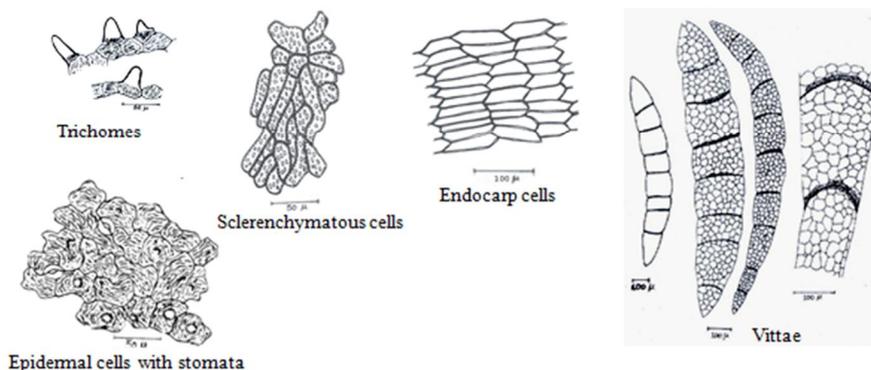


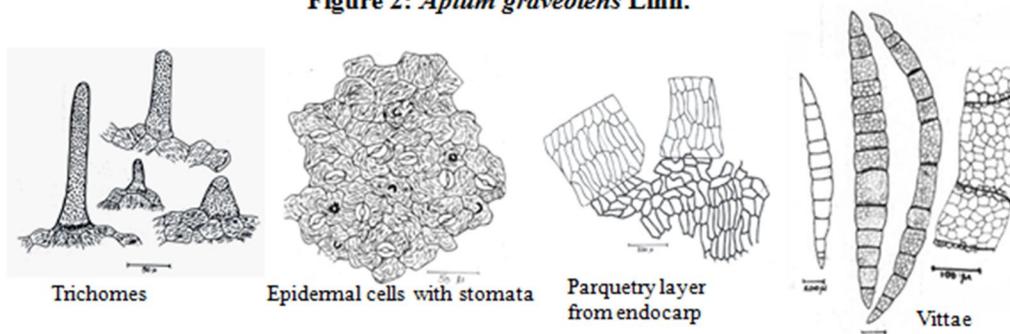
Figure 1: *Apium leptophyllum* (Pers.) F. Muell ex Benth

3.3 *Trachyspermum roxburghianum* (DC.) Craib.

It is an annual herb about 1 m high. Fruits, more mericarps than cremocarps; hispid, with hairs visible to the naked eye; cremocarps are ovate or ovoid – oblong, being longer than broad (Plate I-C); grayish, with straight, brownish conspicuous ridges, base rounded; stylopod conical; valleculeae darker and narrow; each mericarp has three dorsal and two lateral ridges, all of the same length, dorsal surface convex, giving rise to slightly curved or bent mericarps, while almost flat or

with a depression in the middle on the commissural surface; whole cremocarps seen with naked eyes or in transmitted light at approximately 10 to 20 x show an outline with prominent hairs, several of which are round tipped (Plate II-C).

Table 1 & 2 showed comparative differences among these three umbelliferous fruits. Tissues from the vascular bundles and the seeds do not show any distinctive or characteristic feature useful for diagnosis.

Figure 2: *Apium graveolens* Linn.

3.4 TLC/HPTLC studies

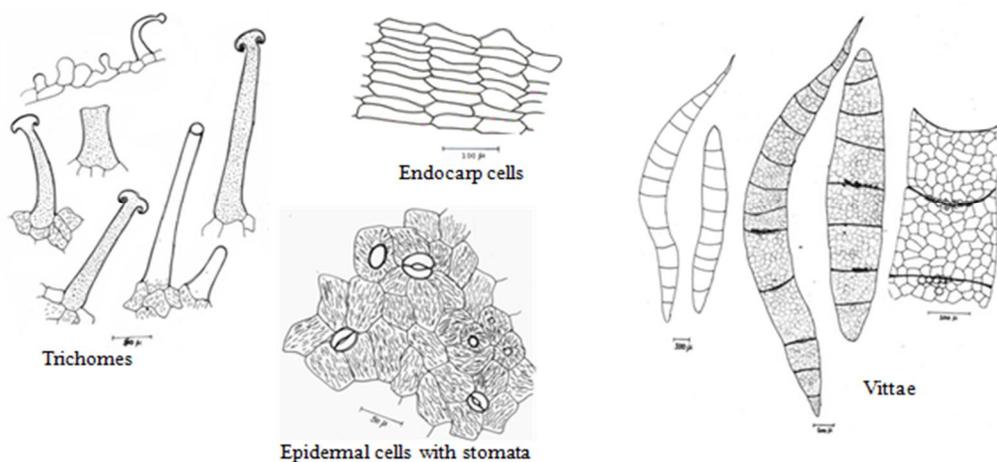
The TLC profile of *n*-hexane extracts of *A.leptophyllum*, *A.graveolens*, *T.roxburghianum* under UV 254 nm is shown in Figure 4. Under UV 254 nm, the *n*-hexane extract of *A.leptophyllum* showed 5 spots at R_f 0.46, 0.52, 0.62, 0.71 and 0.81. The *n*-hexane extract of

A.graveolens showed 6 spots at R_f 0.23, 0.46, 0.52, 0.62, 0.71 and 0.81. The *n*-hexane extract of *T.roxburghianum* showed 6 spots at R_f 0.23, 0.46, 0.52, 0.62, 0.71 and 0.81. There were 5 common spots in *n*-hexane extracts under UV 254 nm for *A.leptophyllum*, *A.graveolens* and *T.roxburghianum* at R_f 0.46, 0.52, 0.62, 0.71 and

0.81. The TLC profile of *n*-hexane extracts of *A.leptophyllum*, *A.graveolens*, *T.roxburgianum* under UV 366 nm is shown in Figure 5. Under UV 366 nm, the *n*-hexane extract of *A.leptophyllum* showed 6 spots at R_f 0.21, 0.25, 0.30, 0.36, 0.54 and 0.62. The *n*-hexane extract of *A.graveolens* showed 7 spots at R_f 0.25, 0.30, 0.36, 0.42, 0.48, 0.54 and 0.62. The *n*-hexane extract of *T.roxburgianum* showed 6 spots at R_f 0.25, 0.30, 0.36, 0.48, 0.54 and 0.62. There were 5 common spots in *n*-hexane extracts under UV 366 nm for *A.leptophyllum*, *A.graveolens* and *T.roxburgianum* at R_f 0.46, 0.52, 0.62, 0.71 and 0.81. The TLC profile of *n*-hexane extracts of *A.leptophyllum*, *A.graveolens*, *T.roxburgianum* after derivatization with vanillin-sulphuric acid reagent is shown in Figure 6. In the derivatized

plate with vanillin-sulphuric acid reagent, the *n*-hexane extract of *A.leptophyllum* showed 6 spots at R_f 0.12, 0.30, 0.41, 0.51, 0.61 and 0.81. The *n*-hexane extract of *A.graveolens* showed 9 spots at R_f 0.12, 0.21, 0.30, 0.41, 0.45, 0.51, 0.61, 0.72 and 0.81. The *n*-hexane extract of *T.roxburgianum* showed 8 spots at R_f 0.12, 0.30, 0.41, 0.45, 0.51, 0.72 and 0.81. There were 5 common spots in *n*-hexane extracts after derivatization with vanillin-sulphuric acid reagent for *A.leptophyllum*, *A.graveolens* and *T.roxburgianum* at R_f 0.12, 0.30, 0.41, 0.51 and 0.81. The corresponding HPTLC finger print profile of *n*-hexane extracts of *A.leptophyllum*, *A.graveolens*, *T.roxburgianum* at λ_{max} 254 nm is shown in Figures 10-12 respectively.

Figure 3: *Trachyspermum roxburgianum* (DC.) Craib



The TLC profile of ethyl acetate extracts of *T.roxburgianum*, *A.graveolens*, *A.leptophyllum* under UV 254 nm is shown in Figure 7. Under UV 254 nm, the ethyl acetate extract of *T.roxburgianum* showed 6 spots at R_f 0.31, 0.61, 0.70, 0.82, 0.87 and 0.93. The ethyl acetate extract of *A.graveolens* showed 7 spots at R_f 0.10, 0.31, 0.61, 0.70, 0.82, 0.87 and 0.93. The ethyl acetate extract of *A.leptophyllum* did not show any spots. There were 6 common spots in ethyl acetate extracts under UV 254 nm for *T.roxburgianum* and *A.graveolens* at R_f 0.31, 0.61, 0.70, 0.82, 0.87 and 0.93. The TLC profile

of ethyl acetate extracts of *T.roxburgianum*, *A.graveolens*, *A.leptophyllum* under UV 366 nm is shown in Figure 8. Under UV 366 nm, the ethyl acetate extract of *T.roxburgianum* showed 9 spots at R_f 0.15, 0.19, 0.27, 0.34, 0.46, 0.55, 0.71, 0.77 and 0.90. The ethyl acetate extract of *A.graveolens* showed 8 spots at R_f 0.15, 0.19, 0.27, 0.34, 0.46, 0.55, 0.71 and 0.90. The ethyl acetate extract of *A.leptophyllum* showed 4 spots at R_f 0.27, 0.46, 0.71 and 0.90. There were 4 common spots in ethyl acetate extracts under UV 366 nm for *T.roxburgianum*, *A.graveolens* and *A.leptophyllum* at R_f 0.27, 0.46, 0.71 and 0.90.

The TLC profile of ethyl acetate extracts of *T.roxburgianum*, *A.graveolens*, *A.leptophyllum* after derivatization with vanillin-sulphuric acid reagent is shown in Figure 9. In the derivatized plate with vanillin-sulphuric acid reagent, the ethyl acetate extract of *T.roxburgianum* after derivatization showed 8 spots at R_f 0.11, 0.15, 0.18, 0.33, 0.44, 0.61, 0.72 and 0.90. The ethyl acetate extract of *A.graveolens* showed 7 spots at R_f 0.11, 0.15, 0.18, 0.33, 0.44, 0.61 and 0.90. The

ethyl acetate extract of *A.leptophyllum* did not show any spots. There were 6 common spots in ethyl acetate extracts after derivatization with vanillin-sulphuric acid reagent for *T.roxburgianum* and *A.graveolens* at R_f 0.11, 0.15, 0.18, 0.33, 0.44, 0.61 and 0.90. The corresponding HPTLC finger print profile of ethyl acetate extracts of *T.roxburgianum*, *A.graveolens*, *A.leptophyllum* at λ_{max} 254 nm is shown in Figures 13-15 respectively.

TLC profile of *n*-Hexane extract of three Umbelliferous fruits

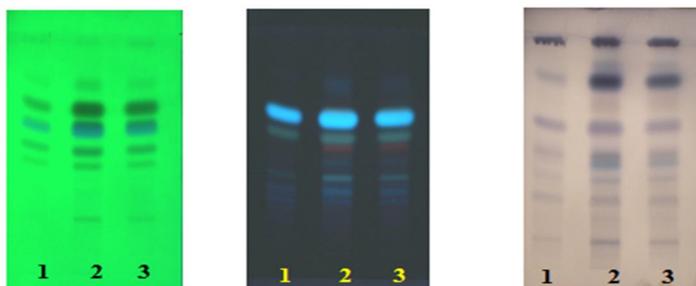


Figure 4: UV 254 nm Figure 5: UV 366 nm Figure 6: Under visible light after derivatization with vanillin-sulphuric acid

1. *Apium leptophyllum*
2. *Apium graveolens*
3. *Trachyspermum roxburghianum*

4. Conclusion

An examination of the various categories of formulations included in the Ayurvedic Formulary of India, shows a wide use of *ajamoda* fruit in many processed formulations. Viz. Ajamodarka and Ajamodadi curna. The

formulations are mostly for internal use, and generally indicate a therapeutic activity in cases of gulma (abdominal lump), aruci (tastelessness), adhmaana, hikka (hiccup), chardi krmi roga, sula (pain of abdomen).

TLC profile of ethyl acetate extract of three Umbelliferous fruits

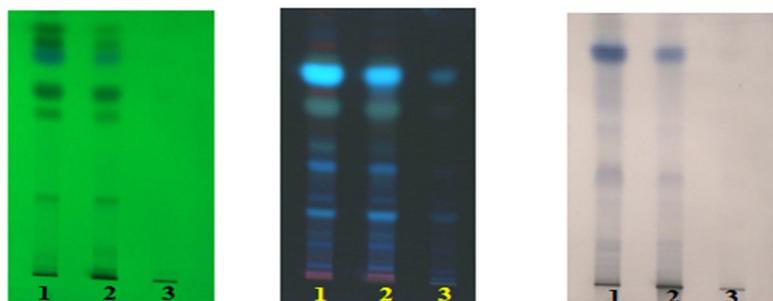
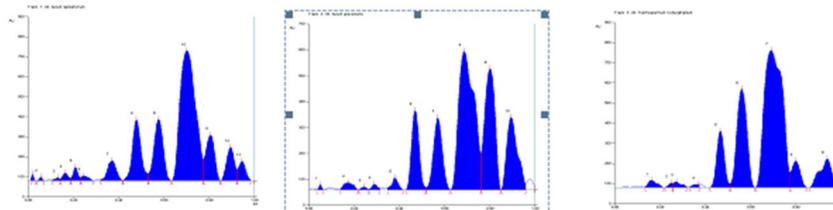
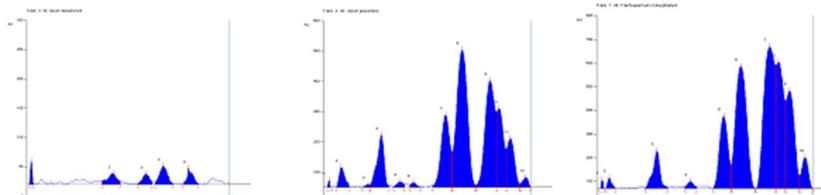


Figure 7: UV 254 nm Figure 8: UV 366 nm Figure 9: Under visible light after derivatization with vanillin-sulphuric acid

1. *Trachyspermum roxburghianum*
2. *Apium graveolens*
3. *Apium leptophyllum*

HPTLC finger print profile of *n*-hexane extract of three Umbelliferous fruitsFigure 10: *Apium leptophyllum* Figure 11: *Apium graveolens* Figure 12: *Trachyspermum roxburghianum*

HPTLC finger print profile of ethyl acetate extract of three Umbelliferous fruits

Figure 13: *Trachyspermum roxburghianum* Figure 14: *Apium graveolens* Figure 15: *Apium leptophyllum*

In the present study the macroscopy as well as microscopic details of the dried three umbelliferous fruits viz. *Apium leptophyllum*, *Apium graveolens*, *Trachyspermum roxburghianum* were studied and described along with TLC and HPTLC finger print profile. The study helps in overcoming the controversy and confusion that exists regarding their proper identity/authenticity and use of one and the same name for more than one drug, and also for identifying the substitute and adulterants. The drug to be used in the name of Ajamoda is *Apium leptophyllum*.

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