

COMPARATIVE MICROSCOPIC, HPTLC AND ANTI-CANDIDAL STUDIES OF *APIUM GRAVEOLENS* L. SEEDS WITH MORPHOLOGICALLY IDENTICAL PLANTS

¹*Rampratap Meena, ²Meera Devi Sri P., ²Mageswari S., ¹Sadaf Subhani and ²Zaheer Ahmed

¹Central Council for Research in Unani Medicine, New Delhi.

²Regional Research Institute of Unani Medicine, Chennai.

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*Corresponding Author

Dr. Rampratap Meena

Central Council for Research
in Unani Medicine, New
Delhi.

ABSTRACT

Apium graveolens L. seed is commonly known as celery seed and belongs to the family Apiaceae. In Traditional system of medicines, the seeds are used for ailment of various diseases. In Unani system of medicine, it is called as Tukhm-e-Karafs and is one of the main ingredient in many pharmacopoeial formulations. But it is often confused with its other closely related spices seeds like *Trachyspermum ammi* (Ajwain), *Trachyspermum roxburghianum* (Radhuni) and *Apium leptophyllum* (Ajamoda) which are more or less similar in appearance. The present paper attempts to explore the

existence of similarity and difference between all the four plant seeds by analyzing its macro microscopical characters, HPTLC finger print profile and its anti-candidal properties to know their potency. From the study, it is concluded that the seeds of *Apium graveolens* are morphologically similar to seeds of *Trachyspermum ammi*, *Apium leptophyllum* and *Trachyspermum roxburghianum* but its microscopical structure and phyto-constituents are different. Since, all samples exhibited anti-candidal activity, other pharmacological activities needs to be thoroughly scrutinized as all the four seeds contains different phyto-constituents.

KEYWORDS: Seeds of Apiaceae; macroscopy; microscopy; HPTLC; Anti-candidal activity.

INTRODUCTION

Seeds of *Apium graveolens* L. are commonly known as celery seeds and Tukhm-e-Karafs in Unani system of medicine. It belongs to the family Apiaceae earlier known as Umbellifereae. In Unani system of medicine, the seeds are used for number of diseases and seem to be one of the constituent of many pharmacopoeial formulations (**Mohammed Tabarak Hussain et al, 2013**). The family Apiaceae has a unique place in homemade remedies and consists of different species of plants ranging from food, fodder, spices, perfumery to few poisonous plants (**Heywood et al, 2007**). The family holds well known economically important plants such as ajwain, asafotida, carrot, coriander, cumin, dill, fennel, parsley etc. *A. graveolens* is one of those spice seed and in Unani system of medicine, the seed, is added as one of the ingredient in many formulations like Banadiqul Buzoori, Dawaul Kurkum Kabir, Habbe Khabsul Hadeed, Jawarish Falafili, Jawarish Safarjali Qabiz, Jawarish Zarooni Sada, Jawarish Zarooni Ambari, Zimad Sumbul Ut Teeb, Sufoof Moya, Sufoof Mohazzil, Majoon Jalali, Majoon Hajrul Yahood, Majoon Dabeedul Ward, Sharbat Bozoori Haar, Majoon Nankhwah etc (**Mohammed Tabarak Hussain et al, 2013**). But, it is very often confused with its other closely related spice seeds like *Trachyspermum ammi* (Ajwain), *Trachyspermum roxburghianum* (Radhuni) and *Apium leptophyllum* (Ajamoda) which are more or less similar in appearance. Upon market and literature survey, there exist quite a lot of confusions in identification of the authentic samples of all the four species of seeds, since all the seeds are available in the name of Ajmod in Hindi (except *Trachyspermum ammi*-Ajwain) and Omam in Tamil. This controversy laid a way to standardize the drug *A. graveolens* along with all the other four plant species.

Thymol (2-isopropyl-5-methylphenol) is the main monoterpene phenol that occurs in few families including Apiaceae family. Literature survey reveals that thymol and carvacarol exhibits good anti bacterial and anti fungal activity (**Anna Marchese et al 2016; Memar Mohammad et al., 2017; Jihong Zhang et al 2019**). Hence efforts were taken to study the sensitivity pattern of all the four seeds against the yeast *Candida albicans*.

The present paper attempts to explore the existence of similarity and difference between all the four plant seeds by analyzing its macro microscopical characters, HPTLC finger print profile and its anti-candidal properties to know their potency.

1. *Trachyspermum ammi* (L.)**Common Name:** Ajwain**Synonyms:** *Trachyspermum copticum* Linn, *Carum copticum* Benth and Hook, *Ammi Copticum* Linn., *Ptychotis coptica* DC and *Lingusticum ajowain* Roxb. The correct generic position is uncertain.**Other Names:** Bishop's weed, carum seed or carum ajowan.**Vernacular Names and Regional Names****English :** Bishops weed**Bengali :** Yamini**Hindi :** Ajwain**Sanskrit :** Dipyaka**Tamil :** Omam**Telugu :** Vamu**Urdu :** Ajowan**Chemical Constituents**

Thymol, carvacrol, p-cymene, γ -terpinene, β -Pinene, p-cymene, γ -terpinene, β -pinene, terpinene-4-ol (**Rafiul Haque *et al.*, 2012**).

Therapeutic Uses

Acts as anti-aggregatory, anti-helmintic, anti-hyperlipidaemic, anti-filarial, insecticidal, kidney stone inhibitory, molluscicidal, mosquito repellent and as nematicidal agent. Administered as a household remedy for stomach disorder, colic pains, asthma etc. (**Sharma L K *et al.*, 2018**).

2. *Trachyspermum roxburghianum* (DC.) Craib**Common Name :** Ajwain**Synonyms :** *Carum roxburghianum* Benth ex Kurz, *Trachyspermum involucreatum* Wolff non Marie, *Athamantha roxburghianum* (Benth.) Wall**Other Names :** Radhuni**Vernacular Names and Regional Names****English :** Wild celery**Bengali :** Radhuni

Hindi	: Ajmud, radhuni, randhuni
Sanskrit	: Vanya ajamoda, Ajamodika
Tamil	: Asamtavomam
Telugu	: Ajumoda, Vamu
Urdu	: Ajmod

Chemical Constituents

D-limonene, α terpinene, dipentene, d-linalool, terpineol, dl-piperitone, thymoquinol, thymol and a crystalline ketonic acid, sabinene, terpinen-4-ol, lingustilide and γ terpinene, apiol, citronellol (Jasim Uddin Chowdhury *et al.*, 2009).

Therapeutic Uses

Acts as carminative, emmenagogue, stimulant and are useful for dyspepsia. Used for bronchitis and asthma (Prathapa Reddy *et al.*, 2018)

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

Common Name: Ajamoda

Synonyms : *Cyclosporum leptophyllum* (Pers.) Sprague ex Britton & P.Wilson. *Apium tenuifolium*, *Cyclosporum ammi*.

Other Names : Marsh parsley, Slender celery, Fir leaved celery

Vernacular Names and Regional Names

English	: Marsh parsley, Slender celery, Fir leaved celery
Bengali	: Randhuni, Banyamani
Hindi	: Ajmuda, Ajmod
Sanskrit	: Dipyaka
Tamil	: Omam
Telugu	: Naranji vamu
Urdu	: Ajmod

Chemical Constituents

Coumarins, D-mannitol, 2,3-dihydro-2-methyl-6-hydroxybenzofuran-5-carboxylic acid, 8-hydroxy cuminic acid, thymohydroquinone dimethyl ether, isothymol methyl ether, thymol methyl ether, cuminaldehyde, p-cymene and γ -terpinene (Iman E Helal *et al.*, 2016).

Therapeutic uses

Acts as carminative, stimulant, cardiac tonic and used to treat bronchitis, asthma, hiccup, and pain in bladder (Saraswathy *et al*, 2013).

4. *Apium graveolens* Linn

Common Name: Karafs

Other Names : Celery

Vernacular Names and Regional Names

Arabic : Bazrul Karafs

Bengali : Chanu, Randhuni

English : Celery

Hindi : Ajmud, Boriajmud

Persian : Karafs, Karasb

Sanskrit : Ajmoda, Brahmakoshi

Tamil : Ashamtagam

Telugu : Ashumagada vomam

Urdu : Ajmod

Chemical Constituents

Coumarines, furanocoumarins (bergapten), flavonoids (apiin) bergapten, limonene, fatty acids, phthalides, β -salinene, salinene, apiol, santalol, sedanolide, isedanic acid, citric, isocitric, fumaric, malic and tartaric acids, oleic, palmitic, paliloleic, petroselinic, petriselaidic, stearic, myristic, myristoleic (Malhotra, 2006).

Therapeutic uses

Acts as stimulant, cardiac tonic, carminative, diuretic, antiseptic, appetizer, purgative. Used to treat spasm, asthma, stomach problems, bronchitis, liver & spleen disorder, expels the stones from kidneys & bladder, sexual debility and for protozoal infestations (Ruchi Roper *et al*, 2017).

2. MATERIALS AND METHODS**Collection of seeds**

The samples sold in the name of Ajwain, Radhuni, Ajamoda and Celery were purchased from different raw drug, local market, Chennai, Tamil Nadu. The drugs were separated based on

their morphology (size, shape and smell) and were subjected to Pharmacognosy and HPTLC studies for correct identification and authentication (**Kirthikar and Basu, 1994; API, 1989,**) of each samples. The voucher specimens were deposited in the raw drug museum DSRU, RRIUM Chennai - DSMTA-83, DSMTR-153; DSMAL-152; DSMAG-81.

Microscopical Study

The microscopical and powder microscopy studies were carried out using standard methods (**Johansen D A, 1940**). The hand sections of the seeds were taken, stained with *safranin* and mounted in *glycerine*. The powder of the drug was treated with various chemical reagents like *phloroglucinol + HCl* and *jeffrey's reagent* for clearing the tissues to study the various elements. Photomicrographs were made using the digital SLR camera attached with the microscope.

HPTLC Studies

Preparation of Extracts for TLC

The seeds of *Trachyspermum ammi*, *Trachyspermum roxburghianum*, *Apium leptophyllum* and *Apium graveolens*, each 5 g were soaked separately in ethanol (20 ml) and allowed to stand for 24hrs with intermittent shaking. The filtrate was collected and concentrated for further HPTLC analysis (**Wagner and Blatt, 1984 and Sethi P D, 1996**).

The extracts (1.0 µl) each were applied over aluminium plate pre coated with silica gel 60 F254 (10x10 cm, E.Merck) by employing CAMAG ATSS\$ sample applicator. The plates were developed upto the distance of 8 cm in the chamber (20x20) consisting of 10 ml of the mobile phase mixture *Toluene: Ethyl acetate (7.8: 2.2)*. The plates were then dried at room temperature, observed and scanned under UV 254 nm & 366 nm. Finally the plates were dipped in vanillin sulphuric acid reagent for a minute and heated at 105°C till the appearance of bright coloured spots.

MICROBIOLOGICAL STUDY

Anti-candidal activity

The in-vitro anti-candidal activity was performed by cup plate method. The *Candida albicans* was procured from National Chemical Laboratory (NCL), Pune. Required number of Muller Hinton agar plates were prepared and swabbed with the log phase culture of the *Candida albicans*. The plates were allowed to dry for few minutes. Wells of about 6mm diameter were cut over the plates at an equidistant position in each plate. The wells were loaded with 60 µl

of increasing concentration of 12.25 mg/ml, 31.5 mg/ml, 62.5 mg/ml, 125 mg/ml, and 250 mg/ml of each drug (alcohol extract of the drug Sample A – *Trachyspermum ammi*; Sample B – *Trachyspermum roxburghianum*; Sample C – *Apium leptophyllum* and Sample D - *Apium graveolens*) respectively to find the Minimum Inhibitory Concentration (MIC). Dimethyl sulphoxide was used as the solvent. All the plates were kept for incubation at 37°C for 24 h.

3. RESULTS AND DISCUSSIONS

1. Macro-Microscopical characteristics

The macro and microscopic characteristics of four plant seeds were tabulated **Table 1**. In terms of macroscopic characteristic, since all the seeds are more or less similar in size, taste and smell acts as the striking feature in identification of four plant seeds. In sectional view, though all are hexagonal in structure, trichomes act as a main character for differentiation among two genus *Trachyspermum* and *Apium*. Both the *Trachyspermum* species shows for the presence of prominent trichomes whereas *Apium* species have reduced or devoid of trichomes **Fig.-1**. The results obtained are similar to the previous studies of (**Pratapa reddy *et al* 2018; Hardel Danendra kumar and Mannem Krishna, 2012**).

Table 1: Macro and Microscopic characteristics of four seeds in comparison.

<i>Trachyspermum ammi</i>	Pale brown in color; ovoid; about 2 mm long and 1.5 mm wide; bitter and pungent taste and more aromatic (thyme odor) due to the presence of thymol; fruit consists of two mericarps each with 5 ridges; cross section shows hexagonal structure; warty trichomes and striated cuticles.
<i>Trachyspermum roxburghianum</i>	Yellowish or greenish brown in color; elongated; elliptical; slightly curved; 2 to 3 mm in length ; strong odor similar to ajwain but taste similar to celery; fruit consists of two mericarps; prominently ridged; cross section shows hexagonal structure with blunt ends and similar warty trichomes with striated cuticles like <i>Trachyspermum ammi</i> .
<i>Apium leptophyllum</i>	Seeds are light to dark brown in color; 1.0 to 1.5 mm long, ellipsoid in shape, the sectional view of the seed shows a wavy outline with minute projections (five ridges); trichomes reduced or absent.
<i>Apium graveolens</i>	Seeds are Dark brown in color; rounded; 2.0 to 3.0 mm in length, the sectional view of the seed shows hexagonal wavy outline. Mericarp have five prominent ridges; reduced trichomes (structure similar to <i>Apium leptophyllum</i>)

Microscopical characteristics

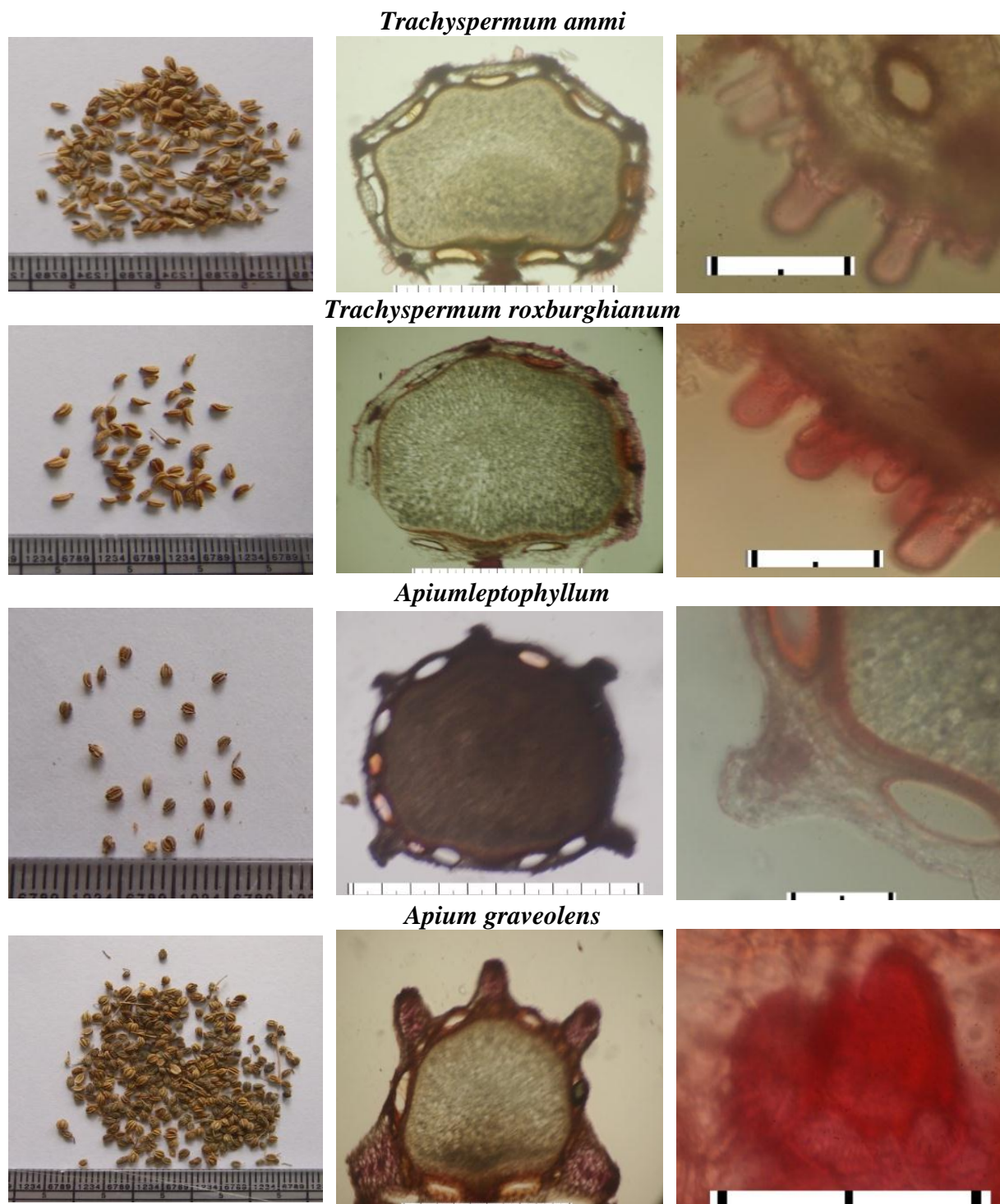


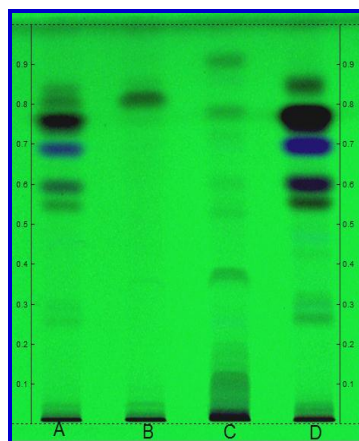
Fig. 1: Macro and Microscopical characteristics of four plant seeds.

1. High Performance Thin Layer Chromatography Studies

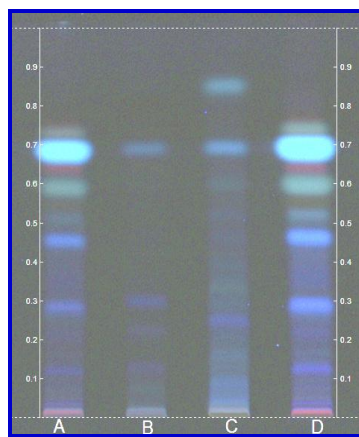
HPTLC profile of Alcohol extracts of all four samples was carried out as per standard methods on 10 cm × 10 cm TLC plates pre-coated with 0.25 μm thin layers of silica gel 60 F₂₅₄ (E. Merck). All four extracts were applied on same plates as bands of 10 mm wide using

an applicator (Linomat V) fitted with a 100 μ L syringe. The application positions of samples (A, B, C & D) were of 10 mm to avoid edge effects. Linear ascending development to a distance of 80 mm with *Toluene: Ethyl acetate: 7.8: 2.2 (v/v)* as mobile phase was performed in a twin-trough glass chamber (20 cm \times 10 cm) previously saturated with vapors of mobile phase for 20 min. The plate was dried in air and visualized under 254 nm and 366 nm for ultra violet detection and taken the fingerprints as evident in **Fig. 2 & 3**. Then, the same TLC plate was derivatized with vanillin-sulphuric acid reagent and visualized in white light and obtained fingerprints as evident in **Fig. 4**. The R_f values of all samples at 254nm and 366nm (absorbance & fluorescence mode) are given in **Table 2**. Under 254nm all the samples show three common bands at R_f 0.57, 0.67 and 0.72 and three common bands at R_f 0.23, 0.54 & 0.63 in fluorescence mode of 366nm. After derivatization the plate was viewed in white light and three common bands at R_f 0.35, 0.52, 0.60, 0.71 were observed. The chromatograms and peaks of all samples at 254nm & 366nm are shown in **Fig. 5-8**.

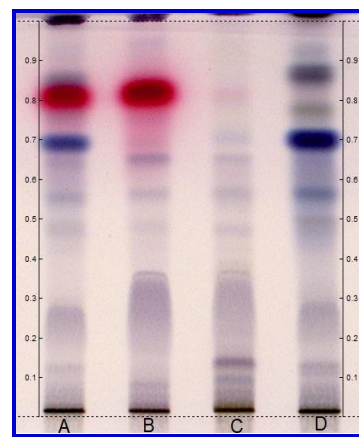
Though the track A and B are for *Trachyspermum* genus and C and D for *Apium* genus, the existence of inter differences in the spots, confirms the differences at species level. Among the Track A (*Trachyspermum ammi*) and B (*Trachyspermum roxburghianum*) there are differences at UV 254 nm and 366 nm, the presence for thymol evidenced by prominent pink spot (VS reagent) confirms the genus as *Trachyspermum*. In the track C (*Apium leptophyllum*), there exists a slight evidence for pink spot (upon VS derivatization) which coincides with the literature for the presence of thymol methyl ether (**Iman E Helal *et al.*, 2016**). The Track D witnessed for the *Apium graveolens* phytoconstituents which may be of coumarines, furanocoumarins, flavonoids (apiin), limonene, apigenin etc., as stated in literature.



UV 254nm
Fig. 2



UV 366nm
Fig. 3



VS derived
Fig. 4

TLC of Four Plant seeds – A: *Trachyspermum ammi* ; B: *Trachyspermum roxburghianum* ; C: *Apium leptophyllum* and D: *Apium graveolens*

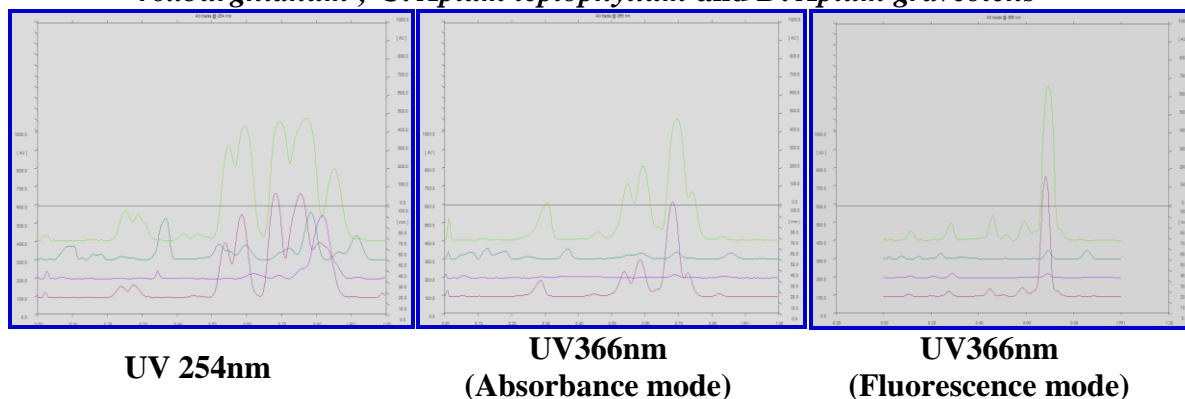


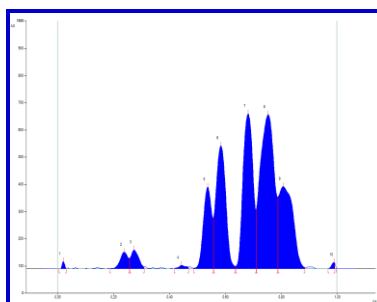
Fig. 5: Densitometry chromatogram of four seeds.

HPTLC chromatogram at UV 254 nm

HPTLC finger print

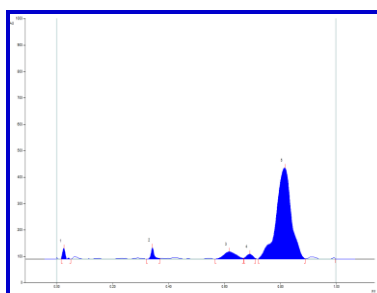
Rf values

Trachyspermum ammi



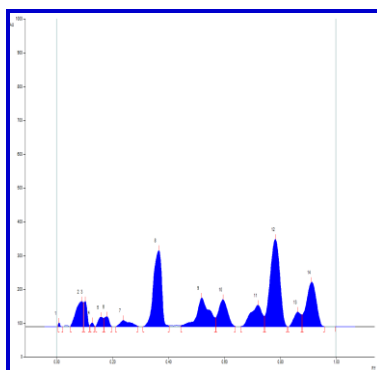
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.02 Rf	25.6 AU	1.07 %	0.03 Rf	1.0 AU	202.4 AU	0.26 %
2	0.19 Rf	0.2 AU	0.24 Rf	60.9 AU	2.56 %	0.26 Rf	38.4 AU	1439.6 AU	1.84 %
3	0.26 Rf	38.9 AU	0.28 Rf	69.2 AU	2.91 %	0.31 Rf	6.5 AU	1597.8 AU	2.04 %
4	0.42 Rf	0.0 AU	0.44 Rf	12.5 AU	0.53 %	0.47 Rf	6.1 AU	264.9 AU	0.34 %
5	0.49 Rf	2.0 AU	0.54 Rf	300.6 AU	12.62 %	0.56 Rf	85.5 AU	7530.7 AU	9.60 %
6	0.56 Rf	188.9 AU	0.59 Rf	452.2 AU	18.99 %	0.64 Rf	8.5 AU	13308.4 AU	16.97 %
7	0.64 Rf	8.6 AU	0.68 Rf	569.0 AU	23.89 %	0.71 Rf	15.2 AU	17277.6 AU	22.03 %
8	0.71 Rf	217.8 AU	0.75 Rf	567.0 AU	23.81 %	0.79 Rf	63.0 AU	23719.5 AU	30.25 %
9	0.79 Rf	263.6 AU	0.81 Rf	301.5 AU	12.66 %	0.89 Rf	4.9 AU	12867.4 AU	16.41 %
10	0.97 Rf	0.1 AU	0.99 Rf	23.1 AU	0.97 %	0.99 Rf	20.8 AU	215.8 AU	0.28 %

Trachyspermum roxburghianum



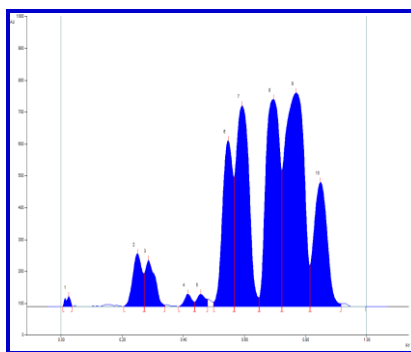
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	2.0 AU	0.03 Rf	40.8 AU	8.58 %	0.05 Rf	0.0 AU	335.4 AU	1.93 %
2	0.32 Rf	0.3 AU	0.34 Rf	43.6 AU	9.17 %	0.37 Rf	2.5 AU	408.4 AU	2.35 %
3	0.57 Rf	0.2 AU	0.62 Rf	27.6 AU	5.80 %	0.67 Rf	3.6 AU	1011.2 AU	5.82 %
4	0.67 Rf	3.8 AU	0.69 Rf	18.6 AU	3.92 %	0.71 Rf	2.7 AU	358.6 AU	2.06 %
5	0.72 Rf	0.6 AU	0.82 Rf	344.9 AU	72.54 %	0.89 Rf	2.0 AU	15274.1 AU	87.84 %

Apium leptophyllum



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	6.6 AU	0.01 Rf	11.1 AU	0.97 %	0.02 Rf	0.0 AU	55.4 AU	0.19 %
2	0.05 Rf	0.4 AU	0.09 Rf	74.3 AU	6.53 %	0.10 Rf	71.4 AU	1629.6 AU	5.58 %
3	0.10 Rf	71.6 AU	0.10 Rf	75.0 AU	6.59 %	0.12 Rf	3.4 AU	744.1 AU	2.55 %
4	0.12 Rf	4.2 AU	0.13 Rf	11.7 AU	1.03 %	0.14 Rf	0.8 AU	95.5 AU	0.33 %
5	0.14 Rf	0.9 AU	0.16 Rf	28.6 AU	2.51 %	0.17 Rf	25.1 AU	419.9 AU	1.44 %
6	0.17 Rf	25.2 AU	0.18 Rf	29.9 AU	2.62 %	0.20 Rf	0.2 AU	420.2 AU	1.44 %
7	0.21 Rf	0.1 AU	0.24 Rf	18.6 AU	1.63 %	0.29 Rf	1.9 AU	565.7 AU	1.94 %
8	0.31 Rf	0.6 AU	0.37 Rf	225.2 AU	19.79 %	0.40 Rf	3.0 AU	5537.9 AU	18.95 %
9	0.45 Rf	3.5 AU	0.52 Rf	85.7 AU	7.53 %	0.57 Rf	26.2 AU	3156.4 AU	10.80 %
10	0.57 Rf	26.6 AU	0.60 Rf	80.0 AU	7.02 %	0.64 Rf	0.8 AU	2155.5 AU	7.38 %
11	0.66 Rf	0.2 AU	0.72 Rf	64.1 AU	5.64 %	0.74 Rf	30.6 AU	2175.4 AU	7.45 %
12	0.75 Rf	31.0 AU	0.78 Rf	258.6 AU	22.72 %	0.83 Rf	0.2 AU	7475.4 AU	25.58 %
13	0.83 Rf	0.0 AU	0.86 Rf	43.2 AU	3.79 %	0.88 Rf	35.2 AU	939.5 AU	3.22 %
14	0.88 Rf	35.5 AU	0.91 Rf	132.1 AU	11.61 %	0.96 Rf	0.0 AU	3848.6 AU	13.17 %

Apium graveolens

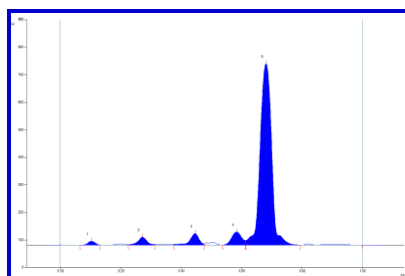


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.03 Rf	31.3 AU	0.95 %	0.04 Rf	1.1 AU	432.2 AU	0.36 %
2	0.21 Rf	0.1 AU	0.25 Rf	166.2 AU	5.07 %	0.27 Rf	03.4 AU	4258.1 AU	3.52 %
3	0.27 Rf	104.6 AU	0.29 Rf	145.1 AU	4.42 %	0.34 Rf	4.9 AU	3915.2 AU	3.23 %
4	0.39 Rf	0.1 AU	0.42 Rf	38.6 AU	1.18 %	0.44 Rf	14.0 AU	768.1 AU	0.63 %
5	0.44 Rf	14.4 AU	0.46 Rf	38.5 AU	1.17 %	0.48 Rf	23.1 AU	888.3 AU	0.73 %
6	0.50 Rf	13.6 AU	0.55 Rf	520.7 AU	15.87 %	0.57 Rf	05.8 AU	14586.1 AU	12.05 %
7	0.57 Rf	407.5 AU	0.59 Rf	629.7 AU	19.20 %	0.65 Rf	27.4 AU	22558.3 AU	18.63 %
8	0.65 Rf	28.9 AU	0.70 Rf	650.1 AU	19.82 %	0.72 Rf	25.8 AU	24898.8 AU	20.56 %
9	0.73 Rf	427.3 AU	0.77 Rf	670.3 AU	20.43 %	0.82 Rf	28.8 AU	34623.1 AU	28.59 %
10	0.82 Rf	130.3 AU	0.85 Rf	389.9 AU	11.88 %	0.92 Rf	9.1 AU	14162.0 AU	11.70 %

Fig. 6: Alcohol extract; Mobile phase: Tolene: Ethyl acetate (7.8:2.2); (1 µl each).

HPTLC chromatogram at UV 366nm

HPTLC finger print

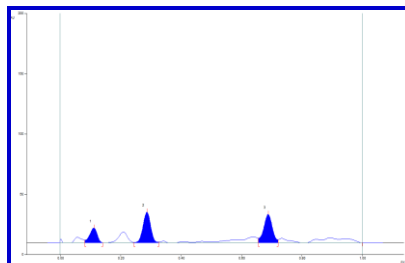


Rf values

Trachyspermum ammi

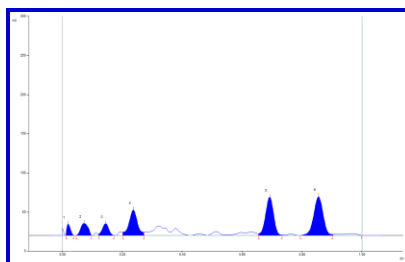
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.0 AU	0.10 Rf	13.7 AU	1.73 %	0.13 Rf	0.0 AU	272.7 AU	1.12 %
2	0.23 Rf	1.8 AU	0.27 Rf	28.9 AU	3.65 %	0.31 Rf	2.2 AU	716.3 AU	2.94 %
3	0.38 Rf	2.5 AU	0.45 Rf	41.9 AU	5.29 %	0.48 Rf	4.1 AU	1061.3 AU	4.35 %
4	0.54 Rf	0.0 AU	0.58 Rf	48.1 AU	6.07 %	0.61 Rf	16.4 AU	1328.3 AU	5.45 %
5	0.62 Rf	16.5 AU	0.68 Rf	659.2 AU	83.26 %	0.80 Rf	0.0 AU	20993.5 AU	86.14 %

Trachyspermum roxburghianum



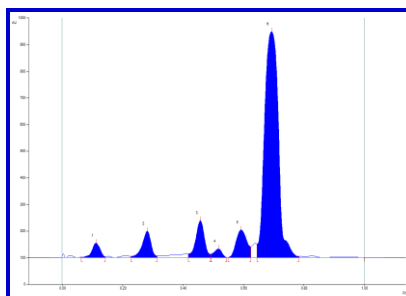
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.08 Rf	2.2 AU	0.11 Rf	12.2 AU	20.00 %	0.14 Rf	0.0 AU	254.5 AU	18.80 %
2	0.24 Rf	0.3 AU	0.29 Rf	25.4 AU	41.57 %	0.33 Rf	0.5 AU	538.9 AU	39.81 %
3	0.66 Rf	3.6 AU	0.69 Rf	23.5 AU	38.43 %	0.72 Rf	2.6 AU	560.2 AU	41.39 %

Apium leptophyllum



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.6 AU	0.02 Rf	14.4 AU	8.20 %	0.04 Rf	0.0 AU	144.8 AU	3.71 %
2	0.05 Rf	0.0 AU	0.07 Rf	15.3 AU	8.72 %	0.10 Rf	0.1 AU	317.4 AU	8.14 %
3	0.12 Rf	1.9 AU	0.15 Rf	15.1 AU	8.57 %	0.17 Rf	0.0 AU	272.6 AU	6.99 %
4	0.20 Rf	4.3 AU	0.24 Rf	32.5 AU	18.49 %	0.27 Rf	4.2 AU	764.0 AU	19.58 %
5	0.66 Rf	2.4 AU	0.69 Rf	49.0 AU	27.84 %	0.74 Rf	0.6 AU	1101.5 AU	28.23 %
6	0.80 Rf	0.0 AU	0.86 Rf	49.5 AU	28.17 %	0.91 Rf	1.3 AU	1301.5 AU	33.36 %

Apium graveolens



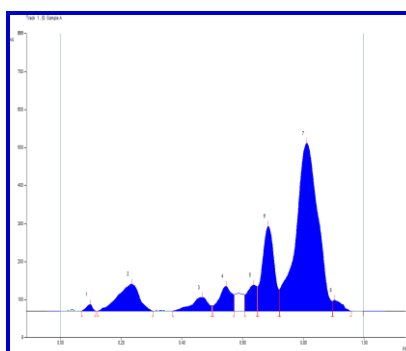
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	1.1 AU	0.11 Rf	54.0 AU	4.24 %	0.14 Rf	3.3 AU	1229.3 AU	2.84 %
2	0.23 Rf	4.7 AU	0.28 Rf	99.1 AU	7.77 %	0.32 Rf	5.7 AU	2427.1 AU	5.61 %
3	0.42 Rf	14.3 AU	0.46 Rf	139.0 AU	10.90 %	0.49 Rf	12.5 AU	3340.9 AU	7.72 %
4	0.49 Rf	12.6 AU	0.52 Rf	32.3 AU	2.53 %	0.55 Rf	0.2 AU	717.8 AU	1.66 %
5	0.55 Rf	0.2 AU	0.59 Rf	103.6 AU	8.12 %	0.63 Rf	40.6 AU	3101.0 AU	7.16 %
6	0.65 Rf	50.9 AU	0.69 Rf	847.7 AU	66.45 %	0.78 Rf	4.0 AU	32474.5 AU	75.02 %

Fig. 7: UV 366 nm (Fluorescence mode).

HPTLC chromatogram After derivatization

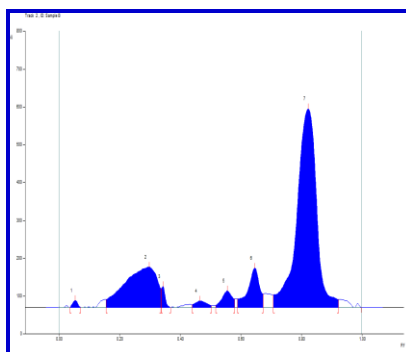
HPTLC finger print

Rf values



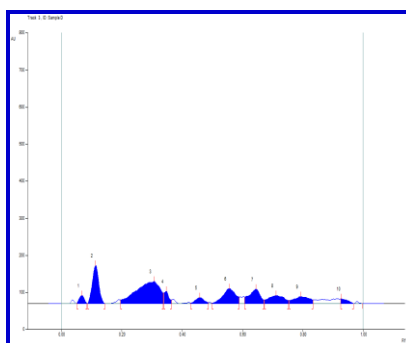
Trachyspermum ammi

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.1 AU	0.10 Rf	17.1 AU	1.80 %	0.12 Rf	0.2 AU	288.6 AU	0.65 %
2	0.12 Rf	0.1 AU	0.24 Rf	70.8 AU	7.44 %	0.31 Rf	0.5 AU	4154.7 AU	9.32 %
3	0.37 Rf	0.5 AU	0.47 Rf	35.9 AU	3.78 %	0.50 Rf	13.7 AU	1626.7 AU	3.65 %
4	0.50 Rf	13.6 AU	0.55 Rf	64.9 AU	6.83 %	0.57 Rf	43.1 AU	2218.5 AU	4.98 %
5	0.61 Rf	42.8 AU	0.64 Rf	68.9 AU	7.25 %	0.65 Rf	64.3 AU	1829.7 AU	4.10 %
6	0.65 Rf	64.5 AU	0.69 Rf	223.0 AU	23.46 %	0.72 Rf	56.2 AU	7256.3 AU	16.28 %
7	0.72 Rf	56.7 AU	0.81 Rf	441.7 AU	46.47 %	0.90 Rf	25.6 AU	26504.2 AU	59.45 %
8	0.90 Rf	25.8 AU	0.90 Rf	28.3 AU	2.98 %	0.96 Rf	0.1 AU	704.9 AU	1.58 %



Trachyspermum roxburghianum

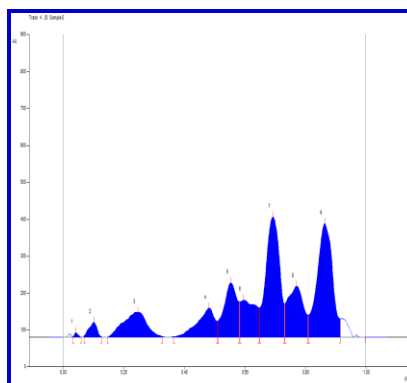
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.2 AU	0.05 Rf	18.1 AU	2.08 %	0.07 Rf	0.2 AU	251.8 AU	0.57 %
2	0.16 Rf	21.6 AU	0.30 Rf	107.2 AU	12.31 %	0.34 Rf	49.3 AU	9436.0 AU	21.52 %
3	0.34 Rf	49.4 AU	0.34 Rf	55.8 AU	6.41 %	0.37 Rf	0.3 AU	577.4 AU	1.32 %
4	0.44 Rf	7.3 AU	0.47 Rf	17.1 AU	1.97 %	0.50 Rf	5.0 AU	560.9 AU	1.28 %
5	0.52 Rf	6.2 AU	0.56 Rf	43.8 AU	5.03 %	0.58 Rf	22.4 AU	1230.9 AU	2.81 %
6	0.59 Rf	22.1 AU	0.65 Rf	104.0 AU	11.94 %	0.68 Rf	35.7 AU	3340.2 AU	7.62 %
7	0.71 Rf	33.4 AU	0.82 Rf	524.6 AU	60.26 %	0.92 Rf	20.7 AU	28451.5 AU	64.89 %



Apium leptophyllum

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.9 AU	0.07 Rf	21.8 AU	5.95 %	0.09 Rf	0.3 AU	301.7 AU	2.64 %
2	0.09 Rf	0.5 AU	0.11 Rf	103.0 AU	28.17 %	0.15 Rf	0.2 AU	1837.9 AU	16.08 %
3	0.20 Rf	9.4 AU	0.31 Rf	59.5 AU	16.26 %	0.34 Rf	28.0 AU	3883.0 AU	33.97 %
4	0.34 Rf	28.1 AU	0.35 Rf	33.4 AU	9.13 %	0.37 Rf	10.1 AU	439.5 AU	3.85 %
5	0.43 Rf	1.9 AU	0.46 Rf	16.1 AU	4.41 %	0.49 Rf	2.4 AU	381.2 AU	3.34 %
6	0.50 Rf	0.8 AU	0.56 Rf	40.4 AU	11.04 %	0.59 Rf	17.6 AU	1411.3 AU	12.35 %
7	0.61 Rf	17.6 AU	0.65 Rf	39.0 AU	10.66 %	0.67 Rf	9.7 AU	1183.1 AU	10.35 %
8	0.67 Rf	9.8 AU	0.71 Rf	21.5 AU	5.88 %	0.75 Rf	8.2 AU	937.3 AU	8.20 %
9	0.76 Rf	8.3 AU	0.79 Rf	18.6 AU	5.09 %	0.83 Rf	10.2 AU	809.1 AU	7.08 %
10	0.93 Rf	12.2 AU	0.93 Rf	12.5 AU	3.42 %	0.97 Rf	0.0 AU	245.9 AU	2.15 %

Apium graveolens



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	1.7 AU	0.04 Rf	12.1 AU	0.99 %	0.06 Rf	0.3 AU	124.1 AU	0.25 %
2	0.07 Rf	3.6 AU	0.10 Rf	40.0 AU	3.28 %	0.13 Rf	0.3 AU	890.9 AU	1.77 %
3	0.15 Rf	0.0 AU	0.25 Rf	67.9 AU	5.58 %	0.33 Rf	1.9 AU	4410.4 AU	8.77 %
4	0.37 Rf	0.8 AU	0.48 Rf	78.7 AU	6.47 %	0.51 Rf	43.3 AU	3745.6 AU	7.45 %
5	0.51 Rf	43.7 AU	0.56 Rf	146.8 AU	12.07 %	0.58 Rf	95.5 AU	5329.2 AU	10.60 %
6	0.58 Rf	95.7 AU	0.60 Rf	100.2 AU	8.23 %	0.65 Rf	79.4 AU	4293.7 AU	8.54 %
7	0.65 Rf	79.7 AU	0.69 Rf	325.2 AU	26.73 %	0.73 Rf	89.7 AU	12402.0 AU	24.67 %
8	0.73 Rf	90.1 AU	0.77 Rf	138.4 AU	11.38 %	0.81 Rf	60.0 AU	5980.7 AU	11.90 %
9	0.81 Rf	60.0 AU	0.87 Rf	307.5 AU	25.27 %	0.92 Rf	49.7 AU	13087.5 AU	26.04 %

Fig. 8: After derivatization with Vanillin sulphuric acid.

Table 2: Rf values of alcohol extract.

S.No.	<i>Apium graveolens</i>	<i>Apium leptophyllum</i>	<i>Trachyspermum ammi</i>	<i>Trachyspermum roxburghianum</i>
254nm	0.21,0.27,0.39,0.44,0.50,0.57,0.65,0.73,0.82	0.12,0.14,0.17,0.21,0.31,0.45,0.57,0.66,0.75,0.83,0.88	0.19,0.26,0.42,0.49,0.56,0.64,0.71,0.79	0.32,0.57,0.67,0.72
366nm Fluorescence mode	0.23,0.42,0.49,0.55,0.65	0.12,0.20,0.66,0.80	0.24,0.66	0.23,0.38,0.54,0.62
After derivatization	0.15,0.35,0.52,0.60,0.65,0.71,0.81	0.20,0.35,0.43,0.52,0.60,0.67,0.71	0.12,0.35,0.52,0.60,0.65,0.71	0.16,0.35,0.44,0.35,0.52,0.60,0.71

3. Anti-candidal Activity

The anti-candidal activity was studied to explore the potency of the four seeds (Alcohol extract), using amphotericin B as standard drug (20 mcg). There are many reports showing the anti-candidal activity of *A. graveolens*, due to presence of phyto-constituent - thymol which is a potent antimicrobial compound. In present study all the seeds exhibited good anti-candidal activity. *A. leptophyllum* and *A. graveolens* exhibited comparatively higher effect against the culture *Candida albicans*. The diameter of zone of inhibition exhibited by *Apium* species was between 26 to 28 mm compared to *Trachyspermum* species which was 15 to 18 mm at the concentration of 250 mg/ml. Comparatively all the seeds are more effective than the standard modern drug amphotericin for *Candida albicans*, the causative agent of many ailments like vaginal problems, UTI, oral thrush, skin infections etc. **Table-3; Fig. 10.**

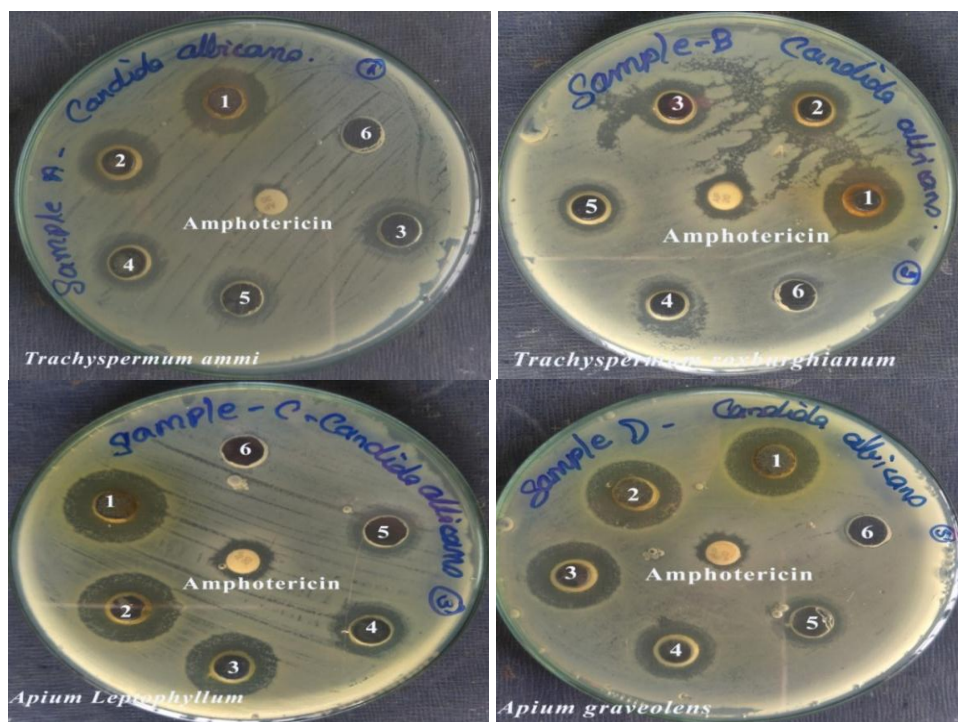


Fig. 9: Anti-candidal activity.

Table-3: Anti-candidal activity of Four seeds (MIC).

Name of the Seeds extract (Alcohol)	Zone of Inhibition in mm						
	1	2	3	4	5	6	Std
<i>Trachyspermum ammi</i>	18	15	13	12	11	-	R
<i>Trachyspermum roxburghianum</i>	17	14	12	11	9	-	R
<i>Apium leptophyllum</i>	27	26	17	13	12	-	R
<i>Apium graveolens</i>	28	27	17	15	12	-	R

Conc: 1: 250mg/ml ; 2 : 125mg/ml ; 3 : 62.5mg/ml ; 4 : 31.2mg/ml ; 5 : 15.6mg/ml
6: DMSO (Vehicle control) and Std: Amphotericin 30mcg ; Well Volume : 60 µl.

CONCLUSION

From the above study, it is concluded that the *Apium graveolens* (celery), *Apium leptophyllum*, *Trachyspermum ammi* and *Trachyspermum roxburghianum* are morphologically similar but microscopical and High Performance Thin Layer Chromatogram studies showed that all four samples are differed from each other. The microbiological activity indicates that *Apium* species exhibits more potency against the organisms than *Trachyspermum* species. Further, it is to mention that each species is different and cannot be taken blindly as substitute based on morphological characters. From the present study, it is concluded that each sample of drug mentioned needs to be checked before conducting of any pharmacological activity.

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