Original Article

Antidermatophytic Activity and Gas Chromatography of Essential Oils

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ABSTRACT

Gas chromatography mass spectrometry (GC-MS) was employed to determine the chemical composition of essential oil obtained from *Eucalyptus globulus* and *Trachyspermum ammi*. The essential oil obtained from leaves of *Eucalyptus globulus* and from seeds of *Trachyspermum ammi* by Clevenger apparatus. Chemical composition of the essential oils of *E. globulus* and *T. ammi* was analyzed by Gas chromatography – Mass spectrometry. Four main compounds in *E. globulus* were identified as Eucalyptol, Methyl – salicylate, Thymol, Para- cymene. 1, 8-cineole was found to be the main compound of *E. globulus* essential oil. Thymol, gamma-terpinene, para- cymene, beta- pinene were the main compounds of *T. ammi* oil and traces amount of beta myrcene, alpha- thujne, alpha-pinene, alpha- terpinene, carvacrol compounds of *T. ammi* were identified by GC-MS. Thymol was the main compound of *T. ammi* oil. The antifungal activity of *E. globulus* and *T. ammi* oils were screened against *Trichophyton mentagrophytes* and *Epidermatophyton floccosum* by using disc diffusion and modified micro dilution method. *T. ammi oil* shows highest inhibition zone in comparison to *E. globulus* oils and references antibiotics i.e. Clotrimazole and Ketoconazole.

Keywords: Gas chromatography – Mass spectrometry, *T. ammi, E. globulus,* Essential oil.

INTRODUCTION

Trichophyton, Microsporum and *Epidermatophyton* are the three genera, which includes all the available dermatophytes[1]. Collectively, they are responsible for ringworm infections in human beings (the Dermatophytosis) [2]. Although there are number of synthetic antifungal are available in the market but they have several side effects[3]. Therefore there is a shift towards natural antifungal for unfolding the folded medicinal importance of some of the important plants which have no side effects[4]. Medicinal plants being natural, non-narcotic, having no side effects, safe, and cost-effective, preventive and

curative therapies. The use of essential oils as functional ingredients in foods, drinks, toiletries, and cosmetics is gaining *momentum*, both for the growing consumers' interest in the ingredients coming from natural sources, and also because of the increasing concern with harmful synthetic additives[5]. Due to their bioactive components, essential oils are indeed promising in view of their use as effective antibacterial, antifungal, and antioxidant agents[6]. *Eucalyptus globulus* is a tall evergreen tree. The leaves and oil of the eucalyptus plant are used for medicinal purposes. *Eucalyptus globulus* oil consists of the volatile oil distilled from the fresh leaves and branch tops of the eucalyptus plant. Topical ointments containing eucalyptus oil have been used in traditional aboriginal medicines to heal wounds and fungal infections[7,8]. The leaves of the eucalyptus plant contain substances that have expectorant, antibacterial and antiseptic properties, but the leaves are also believed to help reduce inflammation and fever. *Eucalyptus globulus* is also known as blue gum eucalyptus. It is a deciduous tree that generally grows from 98 to 180 feet (30 – 55 meter) tall[9,10]. The blue gum eucalyptus typically grows in dense monocultures[11]. The broad juvenile leaves are borne in opposite pairs on square stem. They are about 6 – 15 cm long and covered with a blue-grey, waxy bloom, which is the origin of the common name "blue gum". The mature leaves are narrow, sickle-shaped and dark shining green. They are arranged alternately on rounded stem and range from 15 – 35 cm in length[12]. The bark of the tree shreds often, peeling in large strips. The leaves of *Eucalyptus globulus* are steam-distilled to extract eucalyptus oil. The cineole-based oil is used as component in pharmaceutical preparations such as cough sweets, lozenges, ointments, and inhalants to relieve the symptoms of cold and influenza. *Eucalyptus globulus* oil is used as flavouring. Cineole-based eucalyptus oil is used as flavouring at low level (0.002 %) in various products, including baked goods, confectionery, meat products and beverages[13]. *Trachyspermum ammi* commonly known as 'Ajwain' belonging to family *apiaceae* is distributed throughout India and it is mostly cultivated in Gujarat and Rajasthan[14]. *T. ammi*, a well known spice is traditional herb widely used for curing various diseases in both humans and animals[15]. The plant is used traditionally as stimulant, carminative, flatulence, dyspepsia, diarrhea, abdominal tumors, abdominal pains, piles, and bronchial problems, lack of appetite, galactogogue, asthma and amenorrhoea. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive, cytotoxic activity, Hypolipidaemic, Antihypertensive, ntispasmodic, broncho-dilating action, antilithiasis, diuretic, Abortifacient, Antitussive, Nematicidal, Anthelmintic and Antifilarial activities[16]. Some studies have been carried out on the chemical composition of the essential oil from Ajwain seeds. Aromatic chemicals present in Ajwain; inhibit other undesirable changes in food, affecting its nutritional quality, texture and flavor. *T. ammi* (Ajwain) a major phenolic compound, Thymol is present and has been reported to be an antispasmodic, germicide and antifungal agent[17]. The essential oil of *T. ammi*, the principle active constituents of the oil is phenols, mainly Thymol (35 to 60%) and some carvacrol[18]. Both the phenols Thymol and carvacrol are responsible for the antiseptic, anti-tussive and expectorant properties[19]. The main aim of the present study was to analyze the volatile component of *E. globulus* and *T. ammi* essential oils by Gas chromatography (GC) and gas chromatography mass spectrometry (GC-

MS). Keeping all the above views in mind and as the need of environment, in the present study, a systematic attempt has been made to investigate the potential of *E. globulus* and *T. ammi* as a defensive measurement against superficial infection on human being.

MATERIALS AND METHODS

Collection of *Eucalyptus globulus* leaves and *Trachyspermum ammi* seeds

The leaves of *Eucalyptus globulus* were collected from Eucalyptus tree from Jaipur Ajmer Highway in the month of May to October, 2013. Fresh seeds of T. ammi were purchased from Laxmi Kirana store in M I Road, New market near Bapu Bazaar, Jaipur in the month of July to December, 2102.

Extraction of *Eucalyptus globulus* and *Trachyspermum ammi* Essential Oil

Extraction of oils was carried out by standard hydro distilled method; Clevenger's apparatus was used for extraction of oil. The leaves of the *Eucalyptus* plant were cut with knife and distilled fresh. Fifty grams of the chopped fresh leaves were mixed with 50 ml of distilled water and few pieces of porous earthenware in the distillation flask. As the same, fresh seeds of Ajwain were collected and distilled fresh. Fifty grams of fresh seeds were mixed with 50 ml of distilled water few pieces of porous earthenware in the distillation flask. All the study was carried out at room temperature. Oils were collected from the apparatus and repeat the steps as the desire amount of oils were needed then used the anhydrous sodium sulphate for removal of water traces, after those pure essential oils were stored into dark bottles at 4°C until used. The essential oil thus obtained was subjected to antidermatophytic activity.

Isolation of dermatophytes

The fungi were isolated from waste water samples, which have lots of Keratinophilic debris, by spread plate method on Sabouraud's Dextrose Agar (SDA) media and identified by macroscopic, microscopic and various biochemical tests.

In-vitro Antidermatophytic screening of the oils using disc diffusion method

In vitro antifungal screening was carried out by dice diffusion method. Sterilized Sabouraud's dextrose agar medium were poured into sterilized petriplates and allowed to solidify. Standard size Whatman No. 1filter paper sterilized discs; 6.0mm in diameter were used to determine Antidermatophytic activity. Test inoculm was prepared in 0.9% NaCl solution. The suspension was vortexed properly. Take 1 µl suspension of each fungal isolates, were spread over the marked SDA agar plates. Sterilized filter paper discs were soaked in undiluted (100 %) concentration of single oils and reference antibiotics Clotrimazole (Sigma) and Ketoconazole (Sigma) of 10 mcg/disc concentration). These discs were then placed over the plates preceded with respective microorganisms. The plates were incubated for 4- 5 days at 28ºC. Results of the qualitative screening were recorded as the average diameter of the inhibition zone surrounding the wells containing the test solution. Results were compared with Clotrimazole and Ketoconazole. The MIC was regarded as the lowest concentration that produced a visible zone of inhibition.

Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of the *Eucalyptus globulus* oil and Ajwain oil against *T. mentagrophytes* and *E. floccosum* were determined by Micro dilution method[20] with slight modification. Sterilized Brain heart

infusion agar semisolid agar media were poured into the sterilized culture tubes and allowed to solidify. Test inoculm was prepared in 0.9% NaCl solution, the suspension was vortexed properly. Different concentrations of Ajwain and Eucalyptus oils were added in media containing culture tubes, afterwards a standard platinum loopful (~0.005 ml, Himedia, Flexiloop) of the inoculum suspension was inserted deep into each tube of medium containing a different concentration of oils, as well as a oil-free control. The culture tubes were then incubated at 28°C for 48-72 hours to determine the MIC. MIC was defined as the lowest concentration that did not yield visual growth after the inoculation period. All experiment was performed in triplicate.

Gas chromatography (GC) and Gas chromatography Mass Spectrometry (GC-MS) analysis of E. globulus and T. ammi essential oils

Quantitative and qualitative analysis of the essential oils were performed using a GC-MS apparatus. The analysis was performed with an Rtx 5 MS column. For the GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Nitrogen gas was used as carrier gas with a flow rate of 1.21ml/min. The column was raised from 50ºC to 320ºC at a rate of 3 ºC min. The relative percentage of the oil constituents was expressed as percentage by peak areas and the identification of oil components was based on their retention time with available literature values.

RESULTS AND DISCUSSION

The total ion chromatogram of the essential oil of *E. globulus* is shown on Figure 1. The essential oil was quantified by the peak area normalization method. Table 1 depicts the retention indices, relative percentages and identities of oil constituents. A total of 12 compounds were identified from the retention indices. The major constituents of *E. globulus* were Eucalyptol (24.15%), γ- terpinene (13.44%), Methyl salicylate (12.97%), Thymol (5.1%) and Globulol (0.07%). Our results was coincides with[21] who also reported that monoterpenes as predominant components and 1, 8-cineole as the major compound in the essential oil from *E. globulus.* The essential oil of *T. ammi* was also quantified by peak area normalization method is shown on Figure 2. Table 2 depicts the retention time, relative percentages and identities of oil constituents. A total of 23 compounds were identified from the retention time. Thymol (34.12%), Para- cymene (35.36%), Gamma- terpinene (24.79%) and Beta- pinene were the main constituents of *T. ammi.* Thymol was the main constituent of *T. ammi* essential oil. This study has shown that 1, 8- cineole as the major compound in the essential oil from *E. globulus*. The eucalyptus oil consisted mostly of oxygenated monoterpenes and monoterpenes hydrocarbons, 1, 8 – cineole determines the commercial value of the oil and its significance as a raw material for different industries. As the same results were found by[22] different percentage of 1, 8 – cineole in *E. globulus* leaf oil have been reported in Uruguay (64%), in Cuba, (77%), in California (86.7%), in Morocco (58-82.5%), in Africa (48.7%) and in Argentina (50-60%). Thymol (34.12%) was found to be the major constituent of the Ajwain oil in our findings were similar to[23] reported thymol (36.7%) was found to be the major constituent of the known as ‘ Ajwain- kaphul’ (crude Thymol), while others reported carvacrol as the major constituent of this oil. It has been shown that Thymol and its precursors, cymene and terpinene have strong antimycotic activity. The antidermatophytic activity of essential oils was screened against *T. mentagrophytes* and *E. floccosum*, which are causal organisms of Dermatophytosis or ringworm infection, by

using disc diffusion method shown in table 3&4. *T. ammi* showed excellent antifungal activity(86 mm, MIC 0.3µl/ml against *Trichophyton mentagrophytes* and 63 mm, MIC 0.4µl/ml against *E. floccosum*) while *E. globulus* oil had good antifungal activity (55 mm, 0.6µl/ml against *T. mentagrophytes* and 37 mm, MIC 0.5µl/ml against *E. floccosum*).The inhibition zone of *T. ammi* oil is higher than *E. globulus* oil and reference antibiotics i.e. Clotrimazole (46 mm against *T. mentagrophytes* and 11 mm against *E. floccosum)* and Ketoconazole (63 mm against *T. mentagrophytes,* 10 mm against *E. floccosum)*). Zone of inhibition of single oil (*T. ammi*) was found to be maximum than *Eucalyptus globulus* and reference antibiotics. Our results suggested that *T. ammi* oil must be used as a natural Antidermatophytic agent against fungal infections (Superficial mycosis). Minimum inhibitory concentration of *T. ammi* and *E. globulus* were determined by micro- dilution method shown in table 5, 6, 7 & 8. In our study *T. ammi* essential oil showed the highest antifungal activity against *T. mentagrophytes* and *E. floccosum* our findings coincides with[24] who reported that the essential oil of *Lantana camera L.* was found as the strongest Inhibitor against *T. mentagrophytes*. Our findings also similar with[25] who found Eucalyptus oil showed antifungal activity against *T. mentagrophytes, Microsporum gypseum, T. rubrum* and *E. floccosum*, all the dermatophytes tested with MIC values ranging from 0.4mg/ml to 1.6mg/ml, the minimum fungicidal concentration (MFC) ranged from .8 to 6.4 mg/ml. our results found that the MIC of *T. ammi* oil were range of 0.3 µl/ml to 0.4 µl/ml against *T. mentagrophytes and E. floccosum,* our finding similar with[26] who found the essential oil of *T. ammi* showed strong antifungal activity at 0.1 µl/ml concentration against dermatophytes isolated from clinical trails. Our results proved that *T. ammi* essential oil possess good anti- fungal activity; hence can be used as an antidermatophytic agent in natural medicines for the treatment of skin infections (superficial mycosis).

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REFERENCES

1. Bassiri JS. Epidemiological trends in zoophilic and geophilic fungi in Iran. *Clin. Exp. Dermatol*. 2013; 38: 13-19.
2. Asticcioli S, Silverio A, Sacco L, Fusi I, Vincenti L and Romero E. Dermatophyte infections in patients attending a tertiary care hospital in northern Italy. *New Microbiol*. 2008; 31: 543-548.
3. Tiwari AK, Mishra AK, Kumar A, Srivastava A, Dikshit A, Pandey A and Bajaj AK. A comparative novel method of antifungal susceptibility for *Malassezia furfur* and modification of culture medium by adding lipid supplement. *Journal of Phytology*. 2011; 3: 44-52.
4. Siramon P, Ohtani Y and Ichiura H. Chemical composition and antifungal property of *Eucalyptus camaldulensis* leaf oils from Thialand. *Rec Nat Prod*. 2013; 7: 49-53.
5. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, and Bruni R. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry*. 2005; 91: 621-632.
6. Baratta MT, Dorman HJ, Deans SG, Figueiredo AC, Barroso JG and Ruperto G. Antimicrobial and antioxidant properties of

some commercial essential oils. *Flavour and Fragrance Journal*. 1998; 13: 235-244.
7. Mahdi NK, Gany ZH and Sharief M. Alternative drugs against *Trichomonas vaginalis*. *East Mediter Health J*. 2006; 12: 679-684.
8. Newall CA, Anderson LA and Phillipson JD. Herbal Medicines: A Guid for Health Care Professionals. London, England: pharmaceutical Press. 1996; 72-73.
9. Ashton DH, Gill AM and Groves RH. Fire and the Australian biota. Canberra City, ACT. *The Australian Academy of Sci*. 1981; 339-366.
10. Moral D and Cornelius H. Mechanism of toxacin transport from *Eucalyptus globulus*. *Bulletin of the Torrey Botanical Club*. 1996; 96: 467-475.
11. Skolmen F, Roger G. Growth and yield of some eucalyptus of interest to California. S. Standiford, B. Richard, F. Ledig and Thomas, technical co-ordinator, Proceedings of a workshop on Eucalyptus in California. 1983; 49-57.
12. Moral D and Cornelius H. Mechanism of toxacin transport from *Eucalyptus globulus*. *Bulletin of the Torrey Botanical Club*. 1969; 96: 467-475.
13. Salari MH, Amine G, Shirazi MH, Hafezi R and Mohammed M. Antibacterial effect of *Eucalyptus globulus* leaf extract on pathogenic bacteria isolated from patients with respiratory tract disorder. *Clin. Microbiol. Infect*. 2006; 12: 194-196.
14. Ranjan B, Sodha RS and Rajawat BS. A review Trachyspermum ammi, *Pharmacogn Rev*. 2012; 6: 56-60.
15. Neema R, Khare S, Singh D, Jain P, Pradhan A and Gupta A. GREEN SOURCE: A POWER OF NATURE TO CURE CANCER. *Inter. J. of Phytotherapy*. 2012; 111-116.
16. Ranjan B, Sodha RS and Rajawat BS. A review Trachyspermum ammi, *Pharmacogn Rev*. 2012; 6: 56-60.
17. Nagalakshmi G, Shankaracharya NB and Puranaik J. Studies on chemical and technological aspects of Ajwain (Trachyspermum ammi) syn (Cerum copticum Hiren) seeds. *J. Food Sci. Technol*. 2000; 37: 277- 281.
18. Tsimidou M and Boskou D. Antioxidant activity of essential oils from the plants of the Lamiaceae family, In Spices, Herbs and Edible Fungi, ed, G Charalambous. 1994; 273-283.
19. Treas. and Evans, Pharmacognosy, International 15th edition, saunder Edinburgh, New York. 2002; 25.
20. Provine H and Hadley S. Preliminary evaluation of a semisolid agar antifungal susceptibility test for yeasts and molds. *J. Clin. Microbiol*. 2000; 38: 537-541.
21. Silvestre AJD, Cavaleiro JAS, Delmond B, Filliatre C, Bourgeois G. Analysis of the variation of the essential oil composition of *Eucalyptus globulus* L. from Portugal using multivariate statistical analysis. *Industrial Crops and Products*. 1997; 6: 27-33.
22. Viturro CI. Volatile components of *Eucalyptus globulus* Labill. ssp. bicostata from Jujuy, Argentina. *Journal of Essential Oil Research*. 2003; 15: 206-208.
23. Thangam C and Dhananjayan R. Anti-inflammatory Potential of the Seeds of Carum copticum. *Indian J. Pharmacol*., 2003; 5: 388-391.
24. Amritesh C, Shukla, Lalsangluaii F, Kumar A and Dikshit A. *In vitro* antidermatophytic activity of *Lantana camara L.* against *Trichophyton mentagrophytes* and *T. rubrum*, *Inter. J. of current discoveries and innovations*. 2013; 2: 86-91.
25. Falahati M, Tabrizib NO, Jahaniani F. Antidermatophytic activity of *Eucalyptus camaldulensis* in comparision with *griseofluvin*, *Iranian J. of Pharmacology & Therapeutics*. 2005; 4: 80-83.
26. Sushil KS and Shahi MP. Broad spectrum herbal therapy against superficial fungal infections, International Conference on Natural Resources Engineering & Technology.2006:45-53.

Table 1. Chemical composition of leaf essential oil of *Eucalyptus globules*

Compound	% Composition	Ret. Time
Alpha-pinene	0.37	9.764
Beta-Pinene	0.24	11.62
Myrcene	0.13	12.282
Alpha-Phellandrene	0.27	12.873
Para-cymene	0.81	13.849
Eucalyptol	24.15	14.274
γ- Terpinene	13.44	19.693
Menthol	42.28	21.391
Methyl salicylate	12.97	22.256
Thymol	5.1	26.625
Globulol	0.07	41.234
Beta- Eudesmol	0.16	44.382

Table 2. Chemical composition of seeds essential oil of *Trachyspermum ammi*

Compounds	% composition	Ret. time
Alpha-thujene	0.3476	9.485
Alpha-pinene	0.334	9.767
Camphene	0.0598	10.393
Beta-pinene	2.7642	11.653
Beta-myrcene	0.5698	12.298
Alpha-phellandrene	0.0469	12.891
Trans-beta-Ocimene	0.0551	13.166
Alpha-Terpinene	0.3967	13.475
Para-cymene	35.3642	14.119
Gamma-terpinene	24.7944	15.736
Cis-beta-terpineol	0.0635	15.93
Alpha-terpinolene	0.0961	16.891
Trans-sabinene hydrate	0.1194	17.346
Terpinen-4-ol	0.0375	21.004
Alpha-terpineol	0.1404	22.074
Thymol	34.1215	26.99
Carvacrol	0.4462	27.156
Aromadendrene	0.0479	28.73
Ledol	0.0256	38.706
Epi-gamma-eudesmol	0.0328	40.516
Beta-eudesmol	0.0658	41.227
Trans-nerone	0.0327	50.042
Alpha-naginatene	0.038	52.581

Table 3. Antifungal Activity of *T. ammi* and *E. globulus* oils against *T. mentagrophytes*

Oils	Test Strain	IZ of Samples (mm)	IZ of Standard Ketoconazole	AI	IZ of standard Clotrimazole	AI
T. ammi	*T. mentagrophytes*	86mm	63mm	1.36	46mm	1.86
E. globulus	*T. mentagrophytes*	55mm	63mm	0.87	46mm	1.19

Table 4. Antifungal Activity of *T. ammi* and *E. globulus* oils against *E. floccosum*

Oils	Test Strain	IZ of Samples (mm)	IZ of standard Ketoconazole	AI	IZ of standard Clotrimazole	AI
T. ammi	*E. floccosum*	63mm	10mm	6.3	11mm	5.7
E. globulus	*E. floccosum*	37mm	10mm	3.7	11mm	3.3

IZ = Inhibition zone (in mm) including the diameter of disc (6mm)
AI (Activity index), concentration of oils 100%

Table 5. MIC of Ajwain oil *(T. ammi)* against *Trichophyton mentagrophytes*

Test strain	Different concentration of *T. ammi* oil µl/ml	Growth visually inspected in different concentration of oil
T. mentagrophytes	0.001	0
	0.003	0
	0.006	0
	0.009	+4
	0.01	+4
	0.03	+3
	0.06	+3
	0.09	+2
	0.1	+2
	0.2	+1
	0.3	0
	0.4	0
	0.5	0
	0.6	0
	0.7	0
	0.8	0
	0.9	0
	1.0	0
	Control without oil	100% growth

Growth was measured: +4 excellent growth; +3 best growth; +2 good growth; +1 visible growth; 0 no growth.

Table 6. MIC of Ajwain oil *(T. ammi)* against *Epidermatophyton floccosum*

Test strain	Different concentration of *T. ammi* oil µl/ml	Growth visually inspected in different concentration of oil
E. floccosum	0.001	0
	0.003	0
	0.006	0
	0.009	+4
	0.01	+4
	0.03	+4
	0.06	+3
	0.09	+3
	0.1	+2
	0.2	+1
	0.3	+1
	0.4	0
	0.5	0
	0.6	0
	0.7	0
	0.8	0
	0.9	0
	1.0	0
	Control without oil	100% growth

Table 7. MIC of Eucalyptus oil (*E. globulus*) against *Trichophyton mentagrophytes*

Test strain	Different concentration of *T. ammi* oil µl/ml	Growth visually inspected in different concentration of oil
T. mentagrophytes	0.001	0
	0.003	0
	0.006	0
	0.009	+4
	0.01	+3
	0.03	+3
	0.06	+3
	0.09	+2
	0 .1	+2
	0 .2	+2
	0 .3	+1
	0 .4	+1
	0 .5	+1
	0 .6	0
	0 .7	0
	0 .8	0
	0 .9	0
	1. 0	0
	Control without oil	100% growth

Table 8. MIC of Eucalyptus oil (*E. globulus*) against *Epidermatophyton floccosum*

Test strain	Different concentration of *T. ammi* oil µl/ml	Growth visually inspected in different concentration of oil
E. floccosum	0.001	0
	0.003	0
	0.006	0
	0.009	+4
	0.01	+3
	0.03	+3
	0.06	+3
	0.09	+2
	0.1	+2
	0.2	+1
	0.3	+1
	0.4	+1
	0.5	0
	0.6	0
	0.7	0
	0.8	0
	0.9	0
	1.0	0
	Control without oil	100% growth

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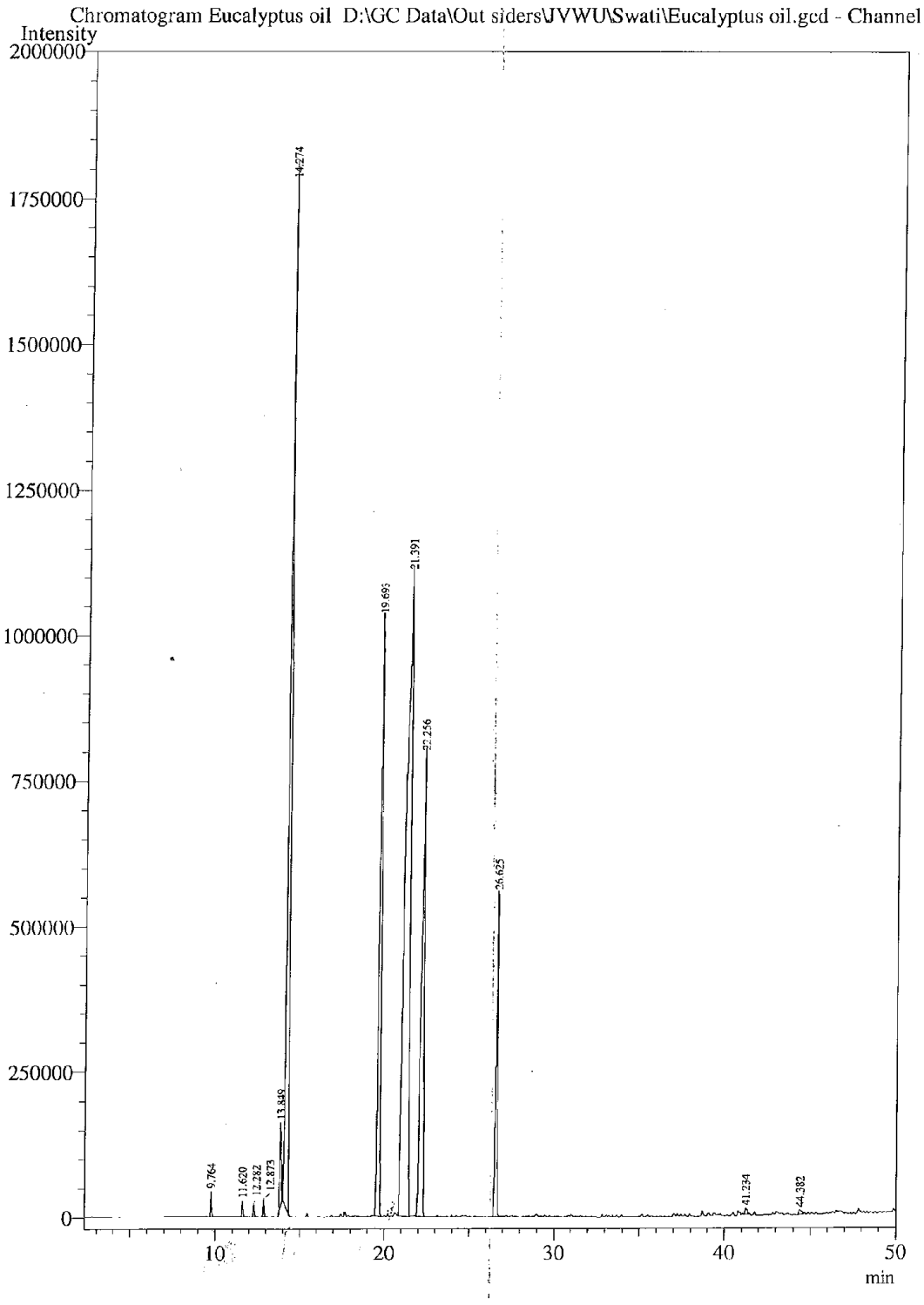

Figure 1. Total ion chromatogram of leaf essential oil of *Eucalyptus globulus* grown in Jaipur, Rajasthan

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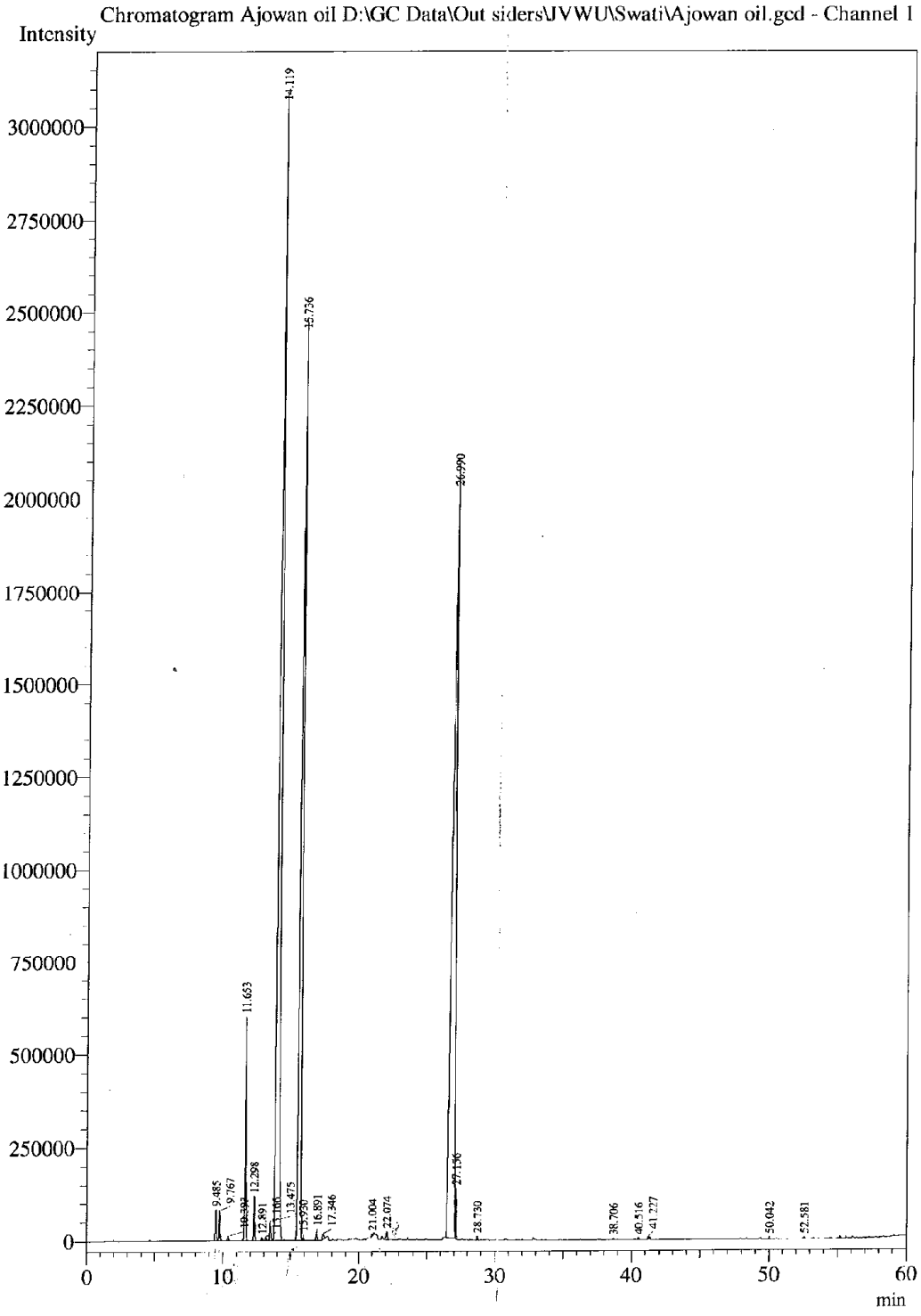

Figure 2. Total ion chromatogram of seeds of essential oil of *Trachyspermum ammi*