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A study on Antidermatophytic Potential of *Ocimum tenuiflorum* Essential Oil and Chemical Composition Evaluation

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Abstract : The Dermatophytes engage in an important role in the atmosphere. They invade the keratinophilic substrates with causing the superficial infections. The certain augmentation in the infection in the human, there is urgent need to search out a new therapeutics or remedies from nature. In the presented study, the chemical composition of *Ocimum tenuiflorum's* volatile oil and its anti-dermatophytic potential was evaluated against isolated Dermatophyte species from Jaipur (India). The Clear pale yellowish colored oil containing 40 volatile components was extracted & analyzed using Hydro Distillation Process and GC & GC-MS methods. The main constitutions were obtained as β-Caryophyllene (38.90%) and Eugenol (19.63%)in the oil and it revealed excellent inhibition activity against test fungal organisms with presence of Maximum Inhibition Zone of 37 mm against *T. mentagrophytes*(KU578106)as well as 31.67 mm for *M. gypsium*and 28.33mmfor *M. nannum* as compared to standard. The results studied in the present research, helps to look out new natural therapeutic drugs from *Ocimum tenuiflorum* as an anti-dermatophytic agent than the standard.
Keywords: Dermatophytes; *Ocimum tenuiflorum;* Volatile; Hydro Distillation; β-Caryophyllene.

Introduction:

Fungi have renowned as a unique division in nature from evolution[1]. In fungi divisions, the *Deuteromycota* Division has awide account of pathogenic fungi in which some have the ability to penetrate the natural keratin and play a role as keratinolytic agent[2-3]. The inventions of fungi cause infection, known as Mycoses[4]. During the last decades, Mycotic infections are raised up to 20–25% in world's population[5]. All these factors have an attempted to search new drugs to treat mycoses.

Plants are an affluent source of organic compounds, many of those are using as drug agents against several infectious and non-infectious diseases, by the modern medicinalsystem[6]. They are livestock, are used by people from Homeopathy, Allopathy, Unani as well as Ayurvedic medicine from Vedic time[7-8].Ayurveda is a "Science of life and longevity" from prehistoric Sanskrit that is one of the remedial systems of India depends on lifestyle, diet, and herbs[9].

The plant's Cytotoxic attitudes are formulated them as anexcellent antiseptic and antifungal agents[10]. There is growing attention in medicinal plants as an alternative source of synthetic molecules with inspiration in the existing marketplace[11]. The *Ocimum tenuiflorum (Krishna Tulsi)* is an aromatic herbaceous plant with 30–60 cm long hairy stems and simple phyllotaxis green or purple leaves & caudiform floral segments that contain Oleanolic acid, linalool, Eugenol, β-elemene and β-caryophyllene as the major constituent of the essential oil. The *Ocimum tenuiflorum* oil can be used as possible pharmaceutical products with therapeutic, perfumery properties[12-14]. On the Basis of remedial activity of *Ocimum tenuiflorum,* in the present study the detailed

chemical composition of its volatile oil and anti-dermatophytic activity was evaluated against *Microsporum species* and *Trichophyton mentagrophytes*(KU578106)species.

Material & Methodology:

- **Collection of Plant material & Extraction of Essential Oil:** The plant material (Leaves & floral) was collected from Jhotwara, Niwaru Road Region, Jaipur and identified as *Ocimum tenuiflorum*. About 500 gm of powdered materials was subjected in Clevenger apparatus hydrodistillation unit on 70ºC–80ºC for 3-5hrs.The extracted pure oil was stored at ±4ºC [15-16].
- **Characterization of Essential Oil:** Identification of pharmacologically active constituents from crude essential oil was characterized by Gas Chromatography (GC) and Gas Chromatography Mass spectrometric (GC/MS) using RTX5MS column. 0.5 µl volumes of each oil samples were automatically injected in the splitless mode. The same volume of N- Hydrocarbons was also automatically injected in the same protocol for linear retention factor. In The GC-MS analysis, the GC program was run in a same occupied column with Helium gas as a carrier at 1.2 ml/min flow rate. Total GC-MS running time was 72.32 min. The detected compounds were identified by processing the raw GC-MS data of Wiley and comparing with National Institute of Standard and Technology (NIST, USA) mass spectral database and from retention times and mass spectra of standard compounds. Relative amounts of detected compounds were calculated based on GC peak areas.
- **Fungal Isolation:** To isolate the clinical fungal species, the skin samples were randomly collected from outdoor patients at the SMS Medical College and Hospital, Jaipur. The KOH Positives were applied on test culture medium and isolates of *Microsporum* sp. (*Microsporum gypseum* and *Microsporum nannum*) and *Trichophyton mentagrophytes* (KU578106)were isolated and identified from to culture via microscopic identification and 18S rRNA sequencing. Sequences are submitted to NCBI Genebank.
- **Inoculums Preparation**: For estimation of antifungal activity, the inoculums were prepared using sterile autoclaved distilled water and mixing it with surface growth, spores and hyphae by a sterile wire loop. The concentration of suspension up to 90% transmittance with approximates 1×106 CFU/ml spores fixed by spectrophotometer at 530 nm.
- **Evaluation of Antifungal assays:**
  The Antifungal activity of the *Ocimum tenuiflorum* was carried out against isolated fungal species of Dermatophytes by the agar diffusion method[17]. The sterile 6.0 mm Standard size Whatman No.1 filter paper discs soaked with *Ocimum tenuiflorum* oil were placed on agar plates containing fungal spore suspension. Respectively Ketoconazole (10mcg/disc) and Fluconazole (10mcg/disc) were also used as a positive control against isolated Dermatophytes. The plates were incubated at 30ºC for 48 to 72 hrs. Three replicates were kept in each case and average values were calculated. The diameter of the inhibition zones (including the diameter of the disk) was measured in mm and the activity index was calculated on the basis of the size of the inhibition zone with analyzing data in mean ± SE via subjected to one-way ANOVA with significant ($P<0.05$).
  In the subsequent method, the different concentrations of essential oil were also diluted series of Pure: 1/2: 1/4: 1/5: 1/7 (Pure: 50: 25: 20: 14%) of essential oil and DMSO solutions. The discs soaked in different concentrations were placed on agar plates containing fungal spore suspension and incubated at 30ºC for 48 to 72 hrs.
- **Determination of Minimum Inhibitory Concentration (MIC)**
  The Minimum inhibitory concentration was evaluated using microdilution method in essential oils using Sabouraud Dextrose Broth Medium[18-20]. In eppendorfs tubes, Different concentrations of *Ocimum tenuiflorum* oil diluted with DMSO ranging in 5: 10: 15: 20: 25: 30: 50: 75: 100µl. Then 0.1 µl inoculums' suspension was inserted deep into each eppendorfs containing broth with different concentration of oil as well as an oil-free control. The tubes were then incubated at 30ºC for 48-72 hours to determine the MIC. MIC was read to be the lowest concentration at which there was no visible growth of the organism by visual inspection.

Result:

The Clear pale yellowish colored oil was extracted from *Ocimum tenuiflorum* with an account of 40 volatile components, representing 96.51% of the total oil which were identified.

Table I: Chemical composition of leaf essential oil of *Ocimum tenuiflorum*

Peak Number	Reaction Time	Area%	Name of Compound	RI	Molecular Weight	Molecular Formula
1.	13.353	0.12	Para Cymene	1022	134	$C_{10}H_{14}$
2.	14.954	0.03	Gamma Terpinene	1056	136	$C_{10}H_{16}$
3.	15.567	0.02	1-Octanol	1069	130	$C_8H_{18}O$
4.	16.358	0.01	Trans Linalool Oxide	1086	170	$C_{10}H_{18}O_2$
5.	17.107	0.02	Nonanal	1102	142	$C_9H_{18}O$
6.	20.068	0.48	Linderol	1165	154	$C_{10}H_{18}O$
7.	20.587	0.17	4-Terpineol	1176	154	$C_{10}H_{18}O$
8.	28.486	0.41	α- Cubebene	1349	204	$C_{15}H_{24}$
9.	29.227	19.63	Eugenol	1366	164	$C_{10}H_{12}O_2$
10.	29.765	2.65	Copaene	1378	204	$C_{15}H_{24}$
11.	30.441	2.27	β-Elemen	1394	204	$C_{15}H_{24}$
12.	31.047	0.49	α-Guaiene	1408	204	$C_{15}H_{24}$
13.	31.925	38.90	β-Caryophyllene	1429	204	$C_{15}H_{24}$
14.	32.602	0.11	β-Barbatene	1445	204	$C_{15}H_{24}$
15.	33.058	2.25	α-Humulene	1456	204	$C_{15}H_{24}$
16.	33.374	0.05	Gamma Amorphene	1464	204	$C_{15}H_{24}$
17.	33.968	0.05	Gamma Muurolene	1478	204	$C_{15}H_{24}$
18.	34.196	2.59	Germacrene-D	1483	204	$C_{15}H_{24}$
19.	34.364	0.09	β-Selinene	1487	204	$C_{15}H_{24}$
20.	34.697	0.55	Cubebol	1495	222	$C_{15}H_{26}O$
21.	35.126	0.24	β-Chamigrene	1506	204	$C_{15}H_{24}$
22.	35.837	0.83	Delta Cadinene	1524	204	$C_{15}H_{24}$
23.	36.595	0.04	β-Calacorene	1543	200	$C_{15}H_{20}$
24.	37.183	0.06	Spathulenol	1558	220	$C_{15}H_{24}O$
25.	38.492	20.39	Caryophyllene Oxide	1592	220	$C_{15}H_{24}O$
26.	38.661	0.50	Viridiflorol	1596	222	$C_{15}H_{26}O$
27.	38.871	0.16	Cedroxyde	1602	220	$C_{15}H_{24}O$
28.	39.286	0.85	Humulene Epoxide Ii	1613	220	$C_{15}H_{24}O$
29.	39.951	0.09	Epicubenol	1630	222	$C_{15}H_{26}O$
30.	40.793	0.12	β-Eudesmol	1653	222	$C_{15}H_{26}O$
31.	40.911	0.21	Gamma Eudesmol	1656	222	$C_{15}H_{26}O$
32.	41.074	0.24	β-Costol	1660	220	$C_{15}H_{24}O$
33.	41.989	0.10	α-Bisabolol	1685	222	$C_{15}H_{26}O$
34.	42.170	0.76	Sesquicineole	1690	222	$C_{15}H_{26}O$
35.	42.931	0.14	α-Costol	1710	220	$C_{15}H_{24}O$
36.	43.281	0.09	Cembrene	1720	272	$C_{20}H_{32}$
37.	43.416	0.20	Larixol	1724	306	$C_{20}H_{34}O_2$
38.	47.590	0.15	Phytone	1843	268	$C_{18}H_{36}O$
39.	55.277	0.20	N-Nonadecanol-1	2081	284	$C_{19}H_{40}O$
40.	56.220	0.18	Phytol Isomer	2111	296	$C_{20}H_{40}O$
		96.51				

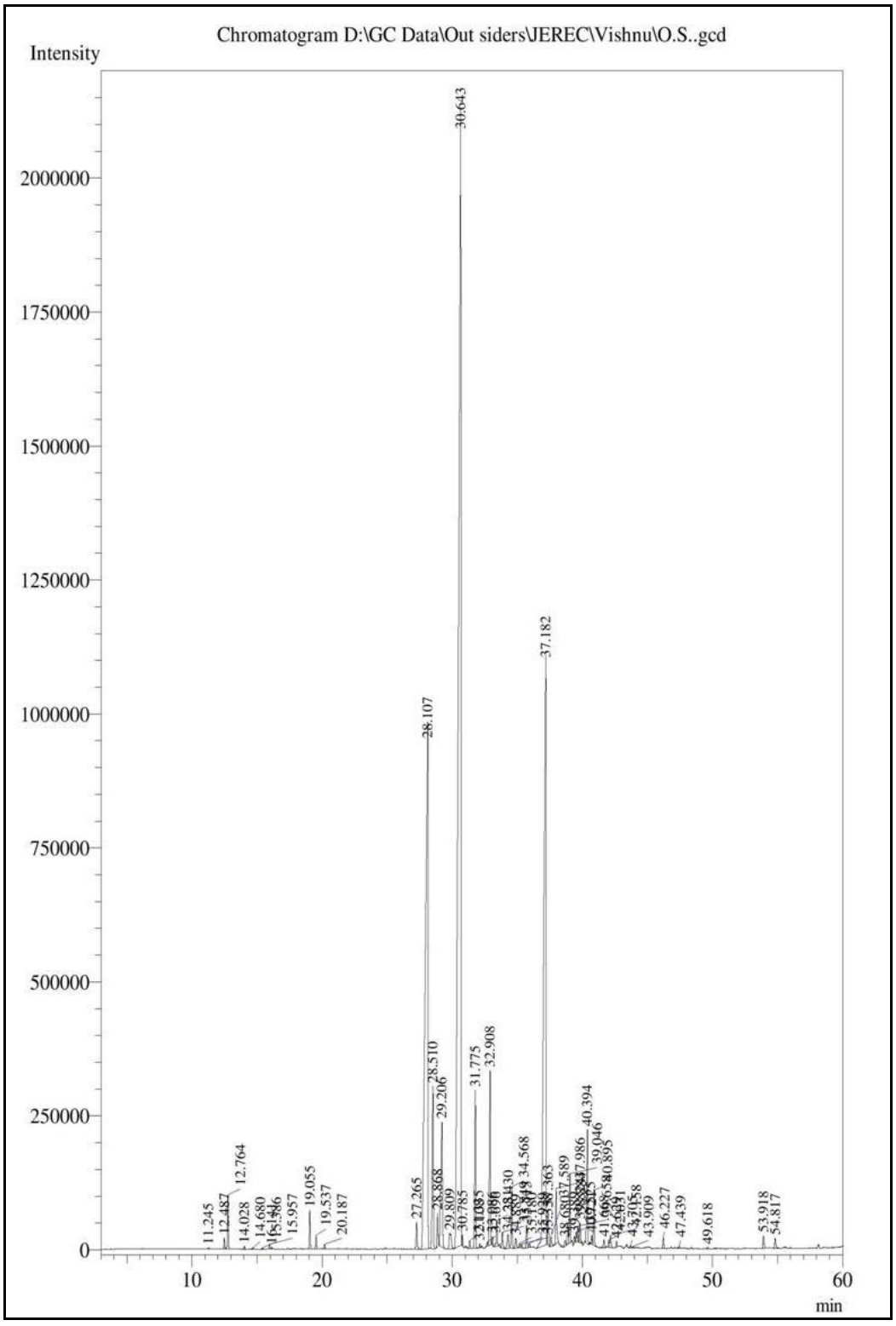

Figure 1: Chromatogram of Essential oil of *Ocimum tenuiflorum* by GC.

The percentage composition and names of the essential oil components are listed in Table-1. The major components of essential oil were β-Caryophyllene (38.90%), Eugenol (19.63%), Caryophyllene-Oxide (20.39%), Copaene (2.65), Germacrene-D (2.59%); β-Elemen (2.27%), α- Humulene (2.25%) and other components were present in trace amounts as presented in Table 1. In Disc diffusion method (Table 2), *Ocimum tenuiflorum* confirmed excellent antidermatophytic activity against selected test fungi. Maximum zone of inhibition was found to be 37mm against *T. mentagrophytes*(KU578106) (Inhibition Zone (IZ): 37mm, Activity

Index (AI): 1.23) as compared to the standard drug. Likewise, the plant oil also revealed excellent activity against *M. gypsium*(IZ: 31.67 mm, AI: 1.17) and *M. nannum*(IZ: 28.33mm, AI: 1.88) as compared to standard.

Table 2: Antifungal activity of *Ocimum tenuiflorum* essential oil with Activity index for Ketoconazole against pathogenic organisms

Test Strain	Antifungal activity of *Ocimum tenuiflorum*				
	IZ of Oil Sample	**IZ of Ketoconazole**	**AI**	**IZ of Fluconazole**	**AI**
***T. mentagrophytes* (KU578106)**	37±1.06	30±3.88	1.23	14±0.70	2.64
M. gypseum	31.67±1.17	20±2.82	1.58	-	-
M. nannum	28.33±3.06	15±0.70	1.88	15.5±1.5	1.83

The concentration of oil used 100%. IZ = inhibition zone (in mm) including the diameter of disc (6 mm), AI = activity index. Experiments were performed in triplicate and data analyzed are mean ± SE subjected to one-way ANOVA with significant (P<0.05).

Ketoconazole is a best antifungal standard against Dermatophytes. Fluconazole was observed in resistant as compared to Ketoconazole. It has resulted only for the *T. menta agrophytes*(KU578106) and*M. nannum*for growth inhibition.The Minimum inhibition activity of *Ocimum tenuiflorum* essential oil was evaluated by modified dilution in oil concentration method against different test fungi. The results showed (Table 2 & 3) that all selected test fungi have good records of resistance activity against neat essential oil (100% concentration) with the formation of inhibition zone. In All Selected fungi, *T. mentagrophytes* (KU578106)shown the highest inhibitory action at 50% oil concentration mixed in DMSO.

Table 3: Inhibition zone activity of *Ocimum tenuiflorum* essential oil at various Dilutions against pathogenic organisms

Inhibition zone activity of *Ocimum tenuiflorum* essential oil at various Dilutions				
Test Fungi	**Oil Dilutions**			
	1/2 (50%)	**1/4 (25%)**	**1/5 (20%)**	**1/7 (14%)**
***T. mentagrophytes* (KU578106)**	22.33±1.17	16.66±1.24	21±1.41	10.30±0.47
M. gypseum	20.9±0.63	15.5±1.76	11.53±0.45	8.63±0.61
M. nannum	21.33±1.65	19±0.70	15.33±1.18	11.33±0.47

The concentration of oil used 100%. IZ = inhibition zone (in mm) including the diameter of disc (6 mm), AI = activity index. Experiments were performed in triplicate and data analyzed are mean ± SE subjected to one-way ANOVA with significant (P<0.05).

For further investigation, the oil was diluted up to 14% concentration and checked against same fungi and observed the zone of 10.30 mm diameter. Similarly,*M. nannum* revealed the results for diluted essential oil concentration of 20% and exhibited highest inhibitory action with 11.33mm inhibition zone.

Table 4: Minimum Inhibition concentration of *Ocimum tenuiflorum* essential oil against pathogenic organisms

Sr. No.	Concentration of oil(in µl/ml)	Test Fungi		
		T. mentagrophytes (KU578106)	***M. gypseum***	***M. nannum***
1.	5	-	-	-
2.	10	+	-	-
3.	15	+	+	+
4.	20	++	+	+
5.	25	++	++	+
6.	30	++	++	+
7.	50	+++	++	++
8.	75	+++	+++	+++
9.	100	+++	+++	+++

- No Inhibition; + Fair Inhibition; ++ Moderate Inhibition: +++ excellent inhibition

The zone size for*T. mentagrophytes* (KU578106)had the highest zone size for 50% value after pure concentration but the Minimum Inhibition activity was observed at 14% concentration with zone size of 10.30mm including thediameter of thedisc. In the present investigation, *T. mentagrophytes*(KU578106)and *M. nannum* were found to be more susceptible fungus at 100% concentration of oil whereas rests of fungi were found to be less sensitive.The MIC Value against all selected strains was observed to start from 10µl/ml concentration as aminimum of essential oil mixed in DMSO. The best observances were recorded from 50µl/ml concentration. At 5-10µl/ml concentrations were observed with fungal growth as well as in Negative control.

Discussion:

In the20th century, the natural remedies have an attraction for strong efficacy, broad spectrum as direct sources of therapeutics, Affordable by the populace, Raw base elaboration up to complex semi-synthetic chemical compounds, Taxonomic markers, Renewable source capability[21-22].The allopathic systems of medicine have less popularity among people that is based on fast therapeutic actions of synthetic drugs. But the traditional route of the healthcare system is returned with herbal medicine that is renowned as "Return to Nature"[23-24]. Plant Essential oils are the best candidature against Dermatophytes[25]. The β-Caryophyllene (38.90%), Eugenol (19.63%),Caryophyllene-Oxide (20.49%), Copaene(2.65), Germacrene-D (2.59%), β-Elemen (2.27%), α- Humulene (2.25%) and other components were present in trace amounts of *Ocimum tenuiflorum* essential oil as presented in Table 1.

The Present study showed the similarity with Joshi and Hoti, 2014 from North West Karnataka, India. In their investigation the major constitutions of *Ocimum tenuiflorum* essential oil were 1, 8-eucalyptol (72.71 %), α-terpineol (2.54 %), terpinen-4-ol (0.34 %), and linalool (0.24 %) were the main methyl Eugenol (82.9%), β-Caryophyllene (4.1%), Borneol (2.4%), Germacrene D (2.3%) and α-Copaene (1.9%) [26]. Similarly again In Belgaum, India, GC-MS analysis was concluded for *Ocimum tenuiflorum* essential oil with *O. gratissimum* oil and reported the major volatile constitution as methyl Eugenol (92.4%) and Eugenol (2.4%), β-Caryophyllene (1.3%). similarly again In Northern India reported the 39 constituents comprising 98% of the oil with themajority of Eugenol (46.2%), (ε)-Caryophyllene (27.6%) and β-Elemene (16.3%) in *Ocimum sanctum* oils[27]. Subsequently In South-east of Borazjan, Iran, 1, 8-Cineole, β-Bisabolen, and Eugenol was found as the main compound in *O. sanctum* oils[28].

The present results of Antidermatophytic activity of *Ocimum tenuiflorum* essential oil by disc diffusion methods showed the agreement with Gupta *et al.*, 2014 who used the plant extract for evaluation of antifungal activity[29]. Here we applied the same procedure to plant essential oil for evaluating the antifungal potential against dermatophytic strains. In present study, researcher observed the excellent antifungal activity as *T. mentagrophytes*(KU578106)(Inhibition Zone (IZ): 37 mm, Activity Index (AI): 1.23), *M. gypsium*(IZ: 31.67 mm, AI: 1.17) and *M. nannum*(IZ: 28.33 mm, AI: 1.88). For MIC Investigation, the Inhibition Activity was showed by the 15µl/ml as lowest value for Minimum inhibition concentration. While the lowest value of using concentration of essential oil as 5µl/ml and10 µl/ml was recorded as negative for MIC evaluation. The 10 µl/ml

concentration of essential oil also showed the imitative MIC Value only for *T. mentagrophytes* (KU578106).In this study, Minimum Inhibition activity for all fungal floras the best Minimum Inhibition activity was for *T. mentagrophytes* (KU578106)of 10.30mm and *M nannum* of 11.33mm at IZ at14% concentration including the diameter of the disc. For *M. gypseum* the best Minimum Inhibition activity was observed at 20% conc. with an inhibition zone of 11.53mm.

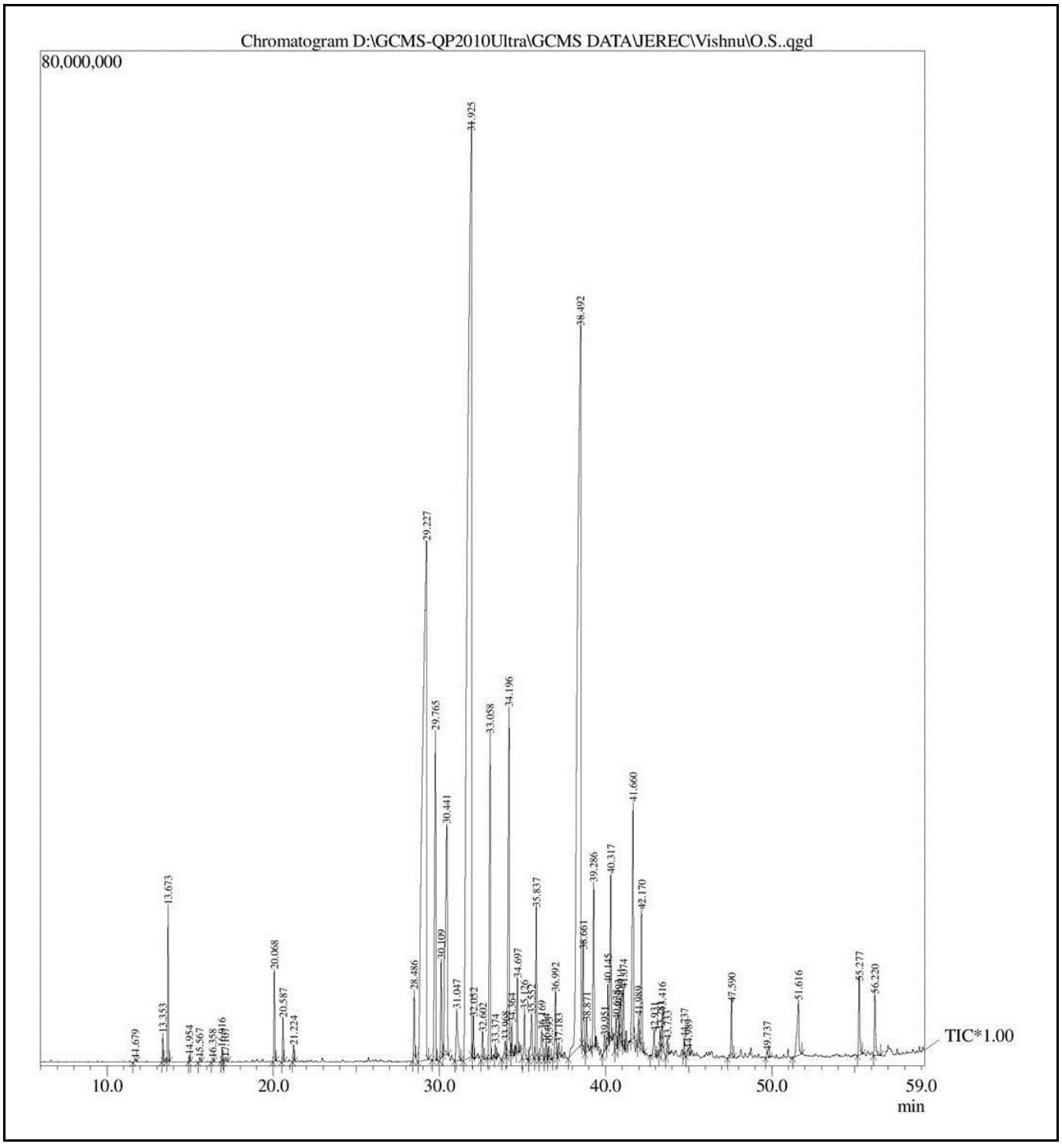

Figure 2: Chromatogram of essential oil of *Ocimum tenuiflorum* by GC-MS.

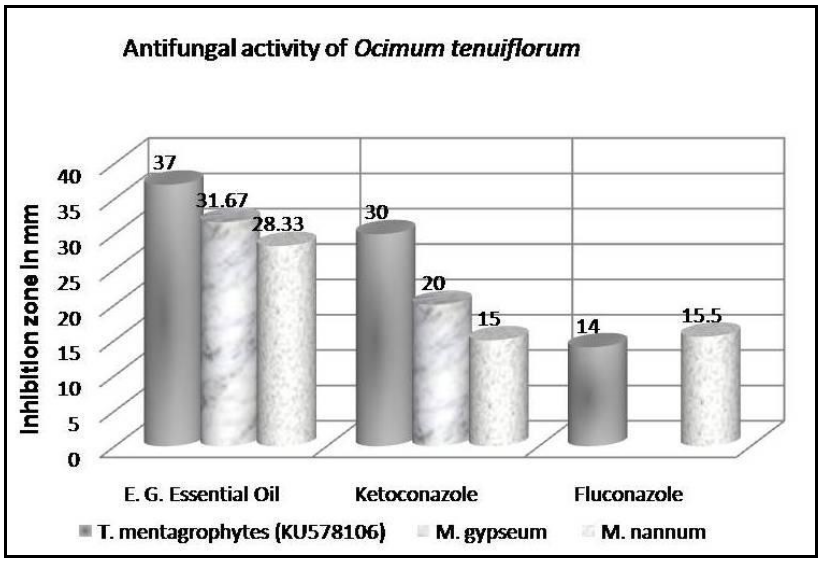

Figure-3: Graphical representation of the comparative Antifungal activity of *Eucalyptus globulus* essential oil with standards against Dermatophytes.

A similar approach was performed by Balakumar *et al.*, 2011 against *T. mentagrophytes*and *E. floccosum* by using disc diffusion method and observed the excellent antifungal activity of *O. sanctum* leaves extracts against clinically isolated dermatophytes at the 200 µg/mL concentration[30]. Another similar study was done by the Silva *et al.*, 2005 in Brazil on Extracts of *Ocimum gratissimum* leaves for in vitro antifungal activity, using agar dilution technique against dermatophytes with a positive 80% inhibition of selective dermatophytes (*Microsporum canis, M. gypseum, Trichophyton rubrum* and *T. mentagrophytes*)[31]. In another study Mahariya and Sharma, 2013 evaluated the *in vitro* antifungal activity against pathogenic fungi by disc diffusion method and microdilution method using the *N sativa* essential oil[32].In their study, resulted in the strong antifungal activity against *Microsporum gypseum, Trichophyton rubrum and Trichophyton simii*with adiameter of inhibition zone and activity index 38 mm (AI: 1.90), 20 mm (AI: 1.33) and 35 mm (AI: 1.09). Sharma et al., 2011 also reported the Minimum inhibitory concentrations (MIC) range between 3.12 -12.5 mg/ml and above for the extracts of the *Solanum melongena* L., *Lawsonia inermis* L. and *Justicia gendarussa* B. against *Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum gypseum* and *Microsporum fulvum* using Agar cup diffusion. Similarly once again, they also observed certain MIC ranging between 0.156-1.25 mg/ml in case of *Piper betle, Allamanda cathertica*[33-34]. Sharma et al., 2012 also reported the positive inhibitions (IZ=7mm and AI=0.19mm) of *O. tenuiflorum* extracts against *A. niger* respectively[35].There are required further studies & analysis to prove the higher antidermatophytic agent of *Ocimum tenuiflorum* as the results obtained higher showed the potential of *Ocimum tenuiflorum* than the standard.

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Conflicts of interest: There are no conflicts of interest to declare for any of the authors in the study.

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