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Research Article

Chemical Profiling And Comparative Analysis Of Ocimum Spp. Essential Oils Using GC-MS

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ABSTRACT

Background & objectives:

A study was performed on the Ocimum plant, to know the chemical composition and significance of the Ocimum species followed by by pharmacognostic study and experimental design with the help of GC-MS. Ocimum genus are very crucial as they have therapeutic potential among the other aromatic herbs.

Methods:

Extreme attention has been put on literature reports in which the utilization of tulsi and their pharmacog- nostic study has been done by performing morphological and microscopic leaf experimental design and by using essential oil through the GC-MS instrumentation method.

Results:

The utilization of these characteristics would be important for the drug discovery scientist to develop a spe- cific formulation of the crude drug, which will be a magical therapeutic agent in the future, with many advantages. GC-MS chromatogram of Ocimum sanctum, Ocimum canum, and Ocimum gratissimum oil showed major peaks and has been identified after comparison of the mass spectra with the NIST library, indicating the presence of three phytocomponents. From the results, the GC-MS study suggested that anethole which is well reported antimicrobial compound is more in O. canum (2.66%) in comparison to O. sanctum (1,28%) but absent in O. gratissimum. The results indicated that the antimicrobial activity is more in O. canum due to the presence ofa high amount of anethole in comparison to O. gratissimum and O. sanctum.

Interpretation & conclusion:

The result revealed that O. canum has a microscopic character that can be identified by the characteristic GC MS analysis of extracts to distinguish between different species of the ocimum plant.

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INTRODUCTION

Holy basil, or Ocimum sanctum L., is a plant of the Lamiaceae family's genus Ocimum that is well-known for its therapeutic properties. Ocimum is one of the most significant fragrant herbs due to its vast medical potential, including its anti-cancer, antidiabetic, spasmolytic, carminative, cardioprotective, anthelmintic, and diaphoretic effects. Ocimum sanctum has a number of biological properties, including anti-inflammatory, hepatoprotective, hypolipidemic, analgesic, and antipyretic properties. Certain phytoconstituents, including luteolin, eugenol, linoleic acid, and β-sitosterol, have been shown to prevent cancers of the skin, liver, mouth, and lungs by enhancing antioxidant activity, triggering apoptosis, changing gene expression, and preventing metastasis11. It has been observed that Ocimum sanctum leaves showed inhibition of the growth of tumour cells10. Ocimum sanctum is an effective medicine so far in inhibiting all kinds of cancer1–4.

Ocimum sanctum

possesses several biological activities with active components such as eugenol, linoleic acid, oleic acid, rosmarinic acid, ocimarin, isori- entin, orientin, aesculectin, aesculin, chlorgrnic acid, ga-luteolin, gallic acid, citronellal, camphene, sabinene, di- methylbenzene, ethylbenzene, vitamin C, and calcium5–7.

MATERIAL & METHODS

Collection, identification, and authentication of selected plants

A collection of all the types of tulsi leaves named Rama Tulsi (Ocimum sanctum), Krishna Tulsi (Ocimum canum), Amrita Tulsi (Ocimum tenuiflorfum), Sweet Basil (Ocimum basilicum), Thai Basil (Ocimum thyrsiflora) and Vana Tulsi (Ocimum gratissimum) of the genus Ocimum were collected for one month from the local area of Haryana, India. Identification of this was confirmed by Dr. RS Jayasomu, Head, Raw material Her- barium and Museum Division (RHMD), NISCAIR, New Delhi, India where a voucher sample (Ref.No. NISCAIR/ RHMD/Consult/-2016/3000-27-2) has been deposited. For further studies in a pharmacognostical manner, phyto- chemical analysis, and extraction, leaves were collected, shade dried, and converted into fine powdered form. inflammation, constipation, and ascites2. It has been utilized to treat malaria-related jaundice and enlarged spleen. It is effective on human nasopharyngeal cancer both in vitro and in vivo in mice. Both the watery extract and the alcohol have high blood pressure. It has antimicrobial and perhaps anticancer properties, and it is cathartic (milky sap). Plant alkaloids are known to have significant biological activity. The leaf of A. cathartic contains alkaloids, sterols, and flavonoids. Strong anti-oxidative cell damage and robust anti-cancer activity are exhibited by flavonoids, which are water-soluble antioxidants and free radical scavengers. Flavonoids have been shown to reduce blood pressure and enhance blood circulation. The flowers of Allamanda cathartica were extracted using acetone, petroleum ether, chloroform, ethanol, and water. Various phytoconstituents, including alkaloids, phenolic substances, saponins, flavonoids, and glycosides, terpenoids, steroids, coumarins, quinones, phytosterols, proteins, and carbohydrates, were found in these extracts. Allamanda cathartica extracts in petroleum ether and chloroform showed encouraging antifungal activity3.

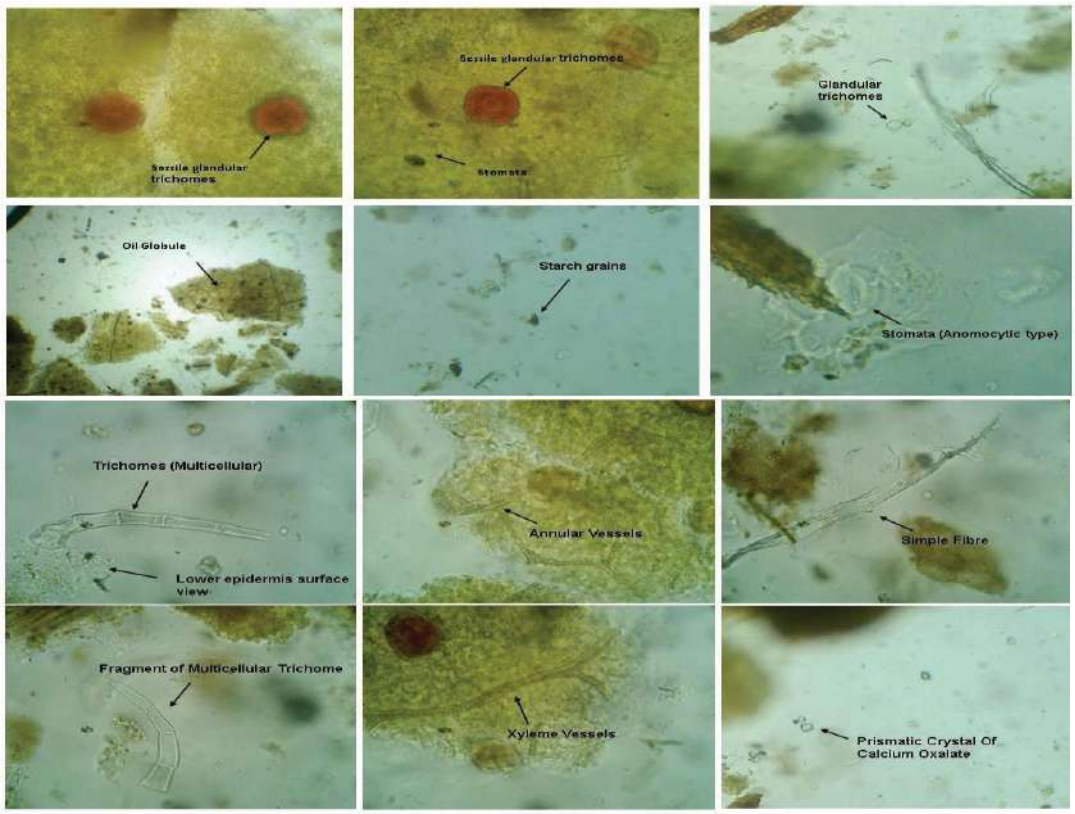

Fig. 1: Powder Microscopy of Rama Tulsi (Ocimum sanctum).

Pharmacognostical study

Following WHO recommendations, morphological and microscopic investigation of the leaf was performed for the pharmacognostic research.

Microscopic studies

Transverse section (TS) of leaf and powder charac- teristics were identified using (Phloroglucinol + HCL) reagents such as chloral hydrate and glycerine to study the cells, fibres, xylem vessels, starch grains, and calcium oxalate crystals. In accordance with Johansen's method, a permanent slide of TS of Leaf was prepared to examine the existence and arrangement of cellular structures. Representative figures were captured using a microscopic imaging camera8.

Experimental design for GC-MS

GC-MS analysis of the Rama Tulsi (Ocimum sanc- tum), Krishna Tulsi (Ocimum canum), Amrita Tulsi (Ocimum tenuiflorfum), Sweet Basil (Ocimum basilicum), Thai Basil (Ocimum thyrsiflora) and Vana Tulsi (Ocimum gratissimum) essential oil was performed using the below-given instruments information.

Thermo Trace 1300GC coupled with Thermo TSQ 800 Triple Quadrupole MS.

For GC - THERMO TRACE 1300 GC For MS - THERMO TSQ 8000

- Software used: XCalibur 2.2SP1 with Founda- tion 2.0SP1
- Column: BP 5MS (30m X 0.25mm, 0.25µm)
- Column Makeup: 5% Phenyl Polysilphenylene- siloxane
- Injector: S (Split)
- Injection volume:2.0µL
- Split Ratio: 20:1
- Injector temp: 250˚C
- MS transfer line temp:230˚C
- Ion source temp: 230˚C
- Mass Range:40-700
- Carrier Flow:1.0ml/min

- Oven Program:

Initial Temp: 50˚C Hold time: 1.0 min Temp 1: 220˚C Hold Time: 5.0 min Rate: 5˚C/min

- Detector: MS TSQ 8000
- Library used: NIST 2.0

Microscopical evaluation

A powder microscopy analysis was conducted on a few types of Tulsi, including Vana Tulsi (Ocimum gratissimum), Rama Tulsi (Ocimum sanctum), and Krishna Tulsi (Ocimum canum), in order to characterize the drug through images in the appropriate order. These images are assessed critically using certain microscopic characteristics (Fig. 1). The drug's characteristics were assessed using photos of Vana Tulsi (Ocimum gratissimum) and Krishna Tulsi (Ocimum canum) in the appropriate order. Specific microscopic characteristics are used to critically examine these images. For this, freehand pieces of the plant material were stained to examine the cells, roots, xylem vessels, starch grains, and calcium oxalate crystals using phenolglucinol and HCl reagents such glycerine and chloral hydrate. To examine the existence and arrangement of cellular structures, a permanent slide of TS of Leaf was made, and representative figures were captured using a microscopic imaging camera (Fig. 2). Krishna Tulsi (Ocimum canum) and Vana Tulsi (Oci- mum gratissimum) were done and evaluated through the below-given pictures in their respective sequence. These pictures were evaluated for microscopic features. For this, Freehand sections of the plant material were stained with Phloroglucinol and HCl reagents such as chloral hydrate and glycerine to study the cells, bre, xylem vessels, starch grains, and calcium oxalate crystals. A permanent slide of TS of Leaf was prepared to see the presence and arrangement of structures of cells, figures were taken with the help of a microscopic image camera (Fig. 3).

A. Characterization of Ocimum

Gas Chromatography-Mass Spectrometry analysis

A Perkin-Elmer gas chromatograph (model 8700), with a flame ionization detector (FID), was used for the chemical analysis of the Rama Tulsi (O. sanctum), Krishna Tulsi (O.canum), Amrita Tulsi (Ocimum tenuiflorfum), Sweet Basil (Ocimum basilicum), Thai Basil (Ocimum thyrsiflora) and Vana Tulsi (O. gratissimum) essential oil. The injector and detector were configured to operate at 220°C and 290°C, respectively. The beginning and final temperatures were maintained for three and ten minutes, respectively, while the column thermostat was started at 80°C and increased to 220°C at a rate of 4°C min-1. Helium served as the carrier gas, flowing at a rate of 1.5 mL min−1. A 100:1 split ratio sample of 1.0 µL was injected. A built-in data-handling program of the apparatus (Perkin-Elmer) was utilized for quantification reasons. As a proportion of the entire peak area, the essential oil content was given12–14 (Fig. 4, 5 & 6) (Table1).

Ethical statement: Not applicable

RESULTS

The hydro-distillation of essential oil from leaf ex-tract of Ocimum sanctum L. yielded pale yellow aromatic oil. GC-MS chromatogram of the Ocimum sanctum, Oci- mum canum, Ocimum tenuiflorfum, Ocimum basilicum, Ocimum thyrsiflora and Ocimum gratissimum oil showed major peaks and has been identified after comparison of the mass spectra with the NIST library, indicating the presence of three phytocomponents. The GC-MS study suggested that anethole which is well reported antimicrobial compound is more in O. canum (2.66%) in comparison to O. sanctum (1.28%) but absent in O. gratissimum and Ocimum thyrsiflora. The results indicated that the antimicrobial activity is more in O. canum due to the presence high amount of anethole in comparison to O. gratissimum, Ocimum thyrsiflora and O. sanctum (Table 4).

CONCLUSION

The summarized information has been focused on the microscopic character of O. canum, with the characteris- tic GC MS analysis of the extracts9, to identify different species of the Ocimum plant. The retention time of each chemical constituent is reported for future identification

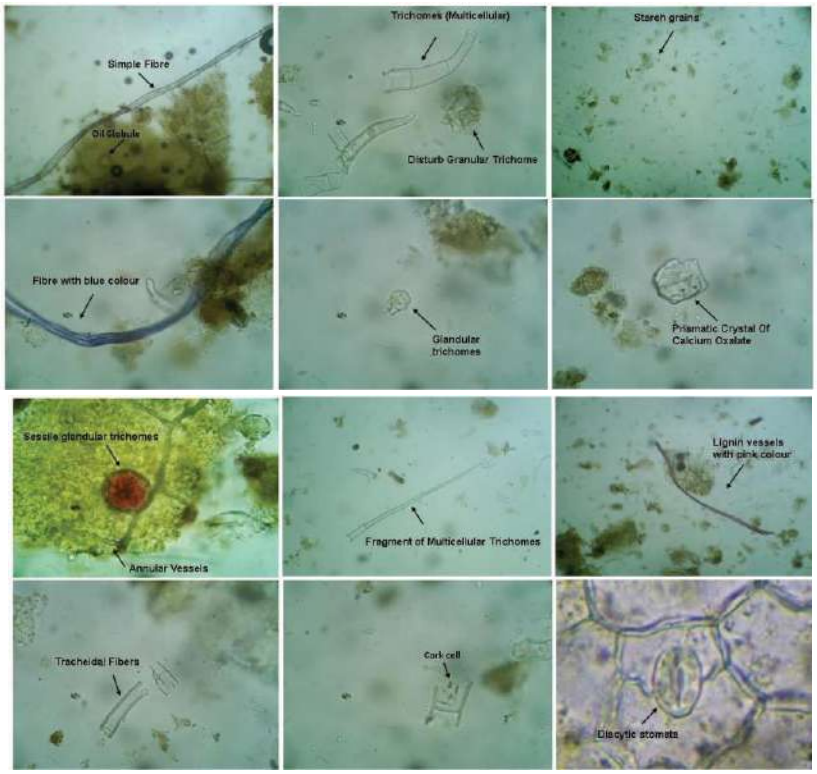

Fig. 2: Powder Microscopy of Krishna Tulsi (Ocimum canum).

Table 1. Chemical Composition of volatile oil extract from O. gratissimum analyzed by GC-MS

RT	Compound Name	Probability	Peak Area	Area %	Molecular Weight
10.75	1,6-Octadien-3-ol, 3,7-dimethyl-	70.62	264140772.25	1.98	154
10.75	Linalyl acetate	5.63	264140772.25	0.36	196
12.36	Linalylisobutyrate	1.68	199242845.32	1.50	224
13.49	1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	1.95	200305251.13	1.50	224
15.17	Estragole	23.88	1042348110.23	7.38	148
17.97	2-Hexyl-1-octanol	3.08	27009602.76	0.20	214

Fig. 3: Powder Microscopy of Vana Tulsi (Ocimum gratissimum).

Table 2. Chemical Composition of volatile oil extract from O. sanctum analyzed by GC-MS

RT	Compound Name	Probability	Peak Area	Area %	Molecular Weight
21.24	1-Dodecanol, 2-octyl-	3.46	136866142.39	0.54	298
21.24	1-Decanol, 2-octyl-	2.95	136866142.39	0.54	270
22.35	2-methyltetracosane	3.26	19645383.15	0.08	352
24.09	Tetracontane, 3,5,24-trimethyl-	4.27	358800273.33	1.41	604
26.82	Octatriacontylpentafluoropropionate	2.09	29166462.68	0.11	696
38.29	Sulfurous acid, butyl octadecyl ester	4.07	1702077568.54	6.70	390

Table 3. Chemical Composition of volatile oil extract from O.tenuiflorfum analyzed by GC-MS

RT	Compound Name	Probability	Peak Area	Area %	Molecular Weight
21.29	1-Dodecanol, 2-octyl-	3.49	136866142.39	0.57	298
21.29	1-Decanol, 2-octyl-	2.99	136866142.39	0.57	270
22.31	2-methyltetracosane	3.24	19645383.15	0.05	352
24.02	Tetracontane, 3,5,24-trimethyl-	4.24	358800273.33	1.49	604
26.88	Octatriacontylpentafluoropropionate	2.06	29166462.68	0.16	696
38.21	Sulfurous acid, butyl octadecyl ester	4.09	1702077568.54	6.77	390

Table 4. Chemical Composition of volatile oil extract from O. basilicum analyzed by GC-MS

RT	Compound Name	Probability	Peak Area	Area %	Molecular Weight
24.01	3,7-Dimethyl-1,6-octadien-3 ol	4.49	136876142.39	0.50	154
20.29	1-Methoxy-4-(prop-2-ene-1-yl) benzene	2.19	136656142.39	0.2	148
21.31	3,7,11-trimethyl-1,3,6,10-dodecatetraene	3.10	19765383.15	0.09	205
24.02	1,8-cineol	4.20	358800273.33	1.49	155
21.88	α-cubebene	1.06	291664682.68	0.16	204
28.21	B-ocimene	3.09	170277568.54	5.77	138

Table 5. Chemical Composition of volatile oil extract from O. thyrsiflora analyzed by GC-MS

RT	Compound Name	Probability	Peak Area	Area %	Molecular Weight
24.01	3,7-Dimethyl-1,6-octadien-3 ol	4.49	136876142.39	0.50	154
20.29	1-Methoxy-4-(prop-2-ene-1-yl) benzene	2.19	136656142.39	0.2	148
20.31	1,2-dimethoxy-4-(prop-2-enyl)benzene	3.21	19765376.15	1.08	178
22.81	α-Bergamotene	1.66	291674682.68	1.16	204
28.21	B-ocimene	3.09	170277568.54	5.77	138

Table 6. Chemical Composition of volatile oil extract from O. canum analyzed by GC-MS

RT	Compound Name	Probability	Peak Area	Area %	Molecular Weight
13.47	1,6-Octadien-3-ol, 3,7-dimethyl	57.31	1415878189.55	4.08	154
13.47	Linalylisobutyrate	1.82	1415878189.55	4.08	224
16.77	Estragole	43.16	10188160501.52	29.39	148
16.77	Benzene, (1-propynylthio)-	0.91	10188160501.52	29.39	148
18.68	10-Methylnonadecane	18.24	57180203.88	0.16	282
18.68	Sulfurous acid, 2-propyl undecyl ester	4.77	57180203.88	0.16	278
19.52	2-Hexyl-1-octanol	8.03	155688172.07	0.45	214
20.07	1-Octadecyne	5.13	55019667.38	0.16	250
20.07	1-Heptadecyne	5.34	55019667.38	0.16	236
22.07	Oxalic acid, cyclobutyloctadecyl ester	6.49	223103118.93	0.64	396
25.50	Levomenol	24.39	321862815.33	0.67	222

of the plant and its variant. It will serve as a literature source in the future to identify variant plant species for high-quality plant production. Ocimum sanctum L. and more research is needed to effectively use essential oil for its intended commercial use.

ABBREVIATIONS

OS = Ocimum. sanctum

O. = Ocimum Genus

COX-1 = Cyclooxygenase-1

Table 7. Morphological Analysis

Parameters	Ocimum gratissimum	Ocimum sanctum	Ocimum canum
Color	Green	Green to purple	Green
Odor	Aromatic smell	Aromatic smell	Aromatic smell
Taste	Pungent taste	Warm Pungent taste	Sharp Pungent taste
Size and Shape	2.5-5 cm long and 1.6-3.2 cm broad, oval, pointed and sharp	2.5-5 cm long and 1.5-3.2 cm broad, oval, elliptical, oblong	2.5-5 cm long and 1-2.5 cm broad Lanceolate to oblong-lanceolate, scattered

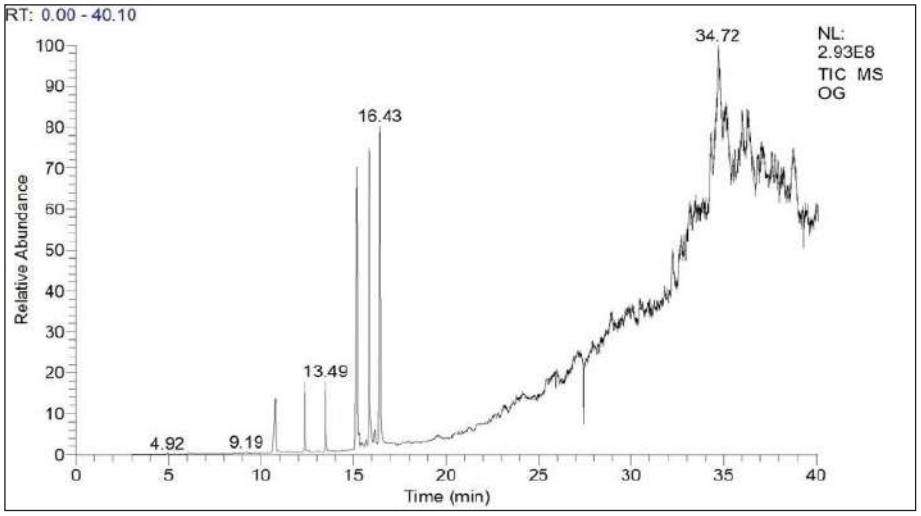

Fig. 4: Characterization of Ocimum gratissimum.

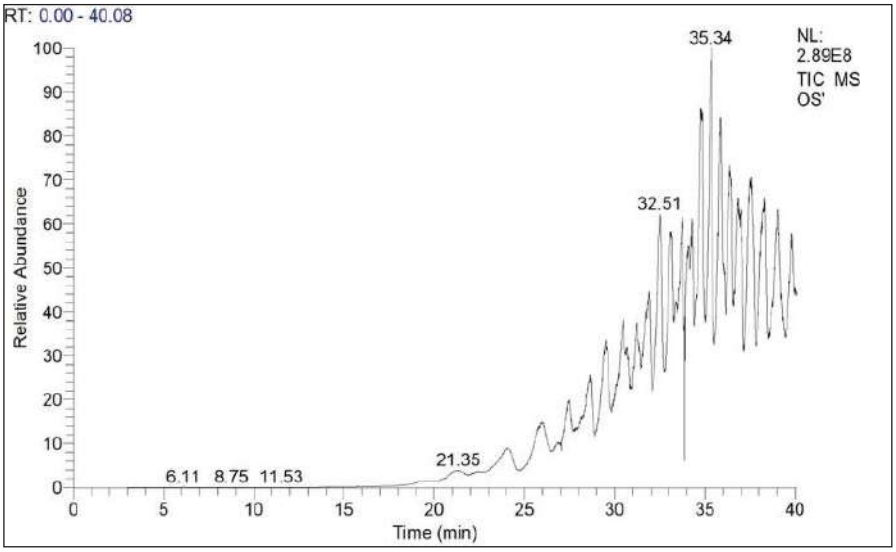

Fig. 5: Characterization of Ocimum sanctum.

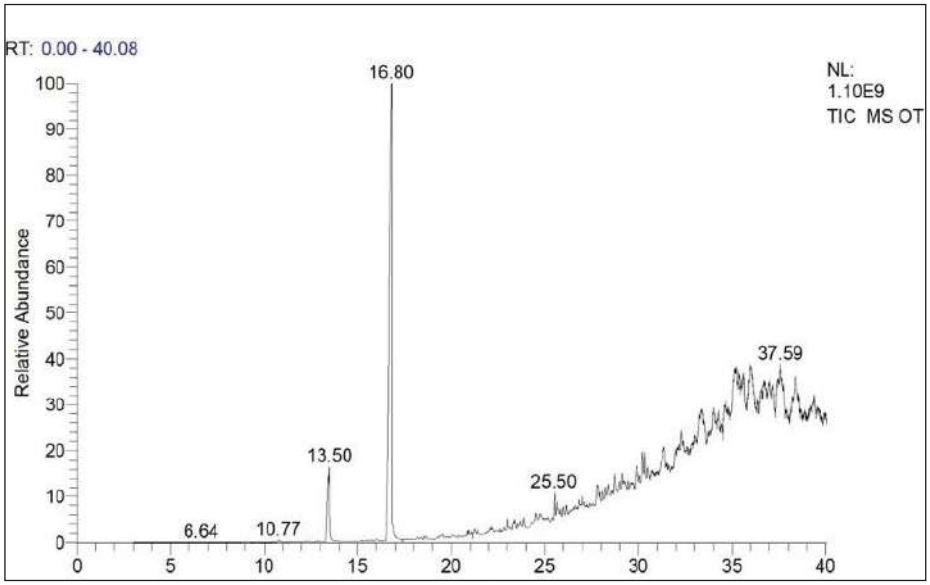

Fig. 6: Characterization of Ocimum canum.

COX-2 = Cyclooxygenase-2
VEGF = Vascular endothelium growth factor
WHO = World Health Organization
COPD = Chronic obstructive pulmonary disease),
IBD = Inflammatory bowel syndrome
DMBA= 7, 12- dimethylbenz(a)anthracene
VEGFR-3 = Vascular Endothelial Growth Factor Recep- tor 3
VLA-4 = Very Late Antigen-4
Conflict of interest: None

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