



Phytochemical analysis of leaf extract of *Ocimum americanum* L. (Lamiaceae) by GCMS method

Shubhangi Nagorao Ingole

Department of Botany, Bar. R.D.I.K. and N.K.D. College Badnera, Amravati, M.S., India

E-mail address: shubhaingole@gmail.com

ABSTRACT

Plants have been an important source of medicine with qualities of thousands of years. *Ocimum americanum* L. belonging to family Lamiaceae is a medicinal and aromatic herb with white or pale liliac flowers in six flowered whorls in 5-10 cm long racemes and having citrus smelled aroma. It is distributed throughout India. The different parts including leaves are used for various medicinal purposes as flavoring agent, diuretic and tonic, for cough, dysentery and relieving toothache. The further studies are conducted to investigate the unexploited potential of *Ocimum americanum*. 13 compounds were identified and they are reported as dibutyl phthalate, 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester, 1,2-benzenedicarboxylic acid, bis (2-methyl propyl ester, 1,2-benzene dicarboxylic acid butyl 2-ethyl hexyl ester; 1,2-benzenedicarboxylic acid butyl 2-methyl propyl ester; phthalic acid; butyl 2-pentyl ester; phthalic acid, butyl hept-4-yl ester, phthalic acid, butyl hex-3-yl ester phthalic acid; Di(2-propyl pentyl) ester diisooctyl phthalate, Bis(2-ethyl hexyl) phthalate; Squalene; Trans-geranylgeraniol. GCMS analysis and the biological activity of each compound were discussed in present attempt. Because of its availability, it can prove as an effective and cheaper drug for various human diseases.

Keywords: GCMS analysis; *Ocimum americanum*; Squalene; Dibutyl phthalate

1. INTRODUCTION

Ocimum americanum L. is a medicinal and aromatic herb belonging to family Lamiaceae with strong citrus smelled aroma, 15-35 cm high, diffuse; branches arise from woody root stock, 4-angled, pubescent, leaves 1.0-3.5 x 0.6-2 cm, ovate, gland dotted, apex acute, base cuneate, margins entire or finely serrulate, flowers white or pale lilac in 6-flowered whorls in 5-10 cm long racemes; bracts up to 0.1 cm long, hairy; calyx campanulate 0.3 cm long in flower, enlarged in fruits, hairy below outside; corolla 0.6 cm long, lips ovate-oblong, upper lip shorter than lower lip. Nutlets subglobose, ovoid, brown, sub-trigonous. It is distributed throughout India. (Flowers and Fruits: June-October)

The leaves are used for flavoring sauces, soups etc. The seeds are considered diuretic and tonic, and are used in the preparation of cooling drinks. A decoction of the plant is taken for coughs, that of leaves for dysentery, it is also used as a mouthwash for relieving toothache, Singh *et. al.* (1983).

Plants have many phytochemicals with various bioactives. Studies have reported that extracts from natural products such as fruits, vegetables and medicinal herbs have positive effects against cancer, compared with chemotherapy and or recent hormonal treatments. Therefore many plants have been examined to identify new and effective antioxidant compounds as well as to elucidate the mechanism of action (Swamy and Tan, 2000). Hence the aim of this study is to determine the phytochemical constituents of *Ocimum americanum* to ascertain the rationale for its use in traditional medicine.

2. MATERIALS AND METHODS

2. 1. Collection of Plant Material

The leaves of *Ocimum americanum* were collected from different habitat of Amravati and Pohra forest.

2. 2. Dry Powder Preparation

The plant leaf sample was dried at room temperature and grind into fine powder.

2. 3. Sample Preparation for GCMS Analysis

About 15 g of powdered material of plant was taken in a clean flat-bottomed glass container and soaked in 150 ml of methanol. The container with its content was sealed and kept for 24 hours. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whattman filter paper. The filtrate (methanolic extract) obtained for the plant was evaporated under ceiling fan.

2. 4. GC-MS Analysis

The GCMS analysis was conducted at the Central Instrumentation Laboratory, Punjab University at Chandigarh. 2 mL aliquot was injected into a fisons GC8000 series coupled to a TSQ8000 MS (Triplequadrapole) mass analyzer. The chromatography was performed by using the DB5-MS column. Injection temperature was 230 °C. Helium flow was 1 mL/min. After a 5 min solvent delay time at 70 °C; the oven temperature was increased at 5 °C/min to

310 °C, 1 min isocratic and cooled to 70 °C, followed by the additional 5 min delay. The ion trace integration was done using the mass lab find target method for the characteristic fragment of assigned peaks.

2. 4. Identification of Components

Interpretation of mass spectrum GCMS was conducted using data base of the Central Instrumentation Laboratory (CIL) spectra Libraries. Spectrum of the unknown component was compared with the spectrum of known components stored in the CIL. The molecular weight, molecular formula and the number of hints used to identify the name of the compound from CIL spectra Libraries were recorded.

3. RESULTS AND DISCUSSION

GCMS analyzed results which include the active principles with their molecular formula are presented in Table 6. 13 compounds were identified and they are reported as dibutyl phthalate, 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester, 1,2-benzenedicarboxylic acid, bis(2-methyl propyl ester, 1,2-benzene dicarboxylic acid butyl 2-ethyl hexyl ester; 1,2-benzenedicarboxylic acid butyl 2-methyl propyl ester; phthalic acid; butyl 2-pentyl ester; phthalic acid, butyl hept-4-yl ester, phthalic acid, butyl hex-3-yl ester phthalic acid; Di(2-propyl pentyl) ester diisooctyl phthalate, Bis(2-ethyl hexyl) phthalate; Squalene; Trans-geranylgeraniol.

On further study of each compound, it was found that they individually have its own biological importance. 1,2-Benzenedicarboxylic acid and diisooctyl phthalate are known to possess antimicrobial and antifouling activity. Dibutyl phthalate possess antimicrobial and antifouling properties.

Squalene is a naturally occurring polyprenyl compound primarily known for its key role as an intermediate in cholesterol synthesis. It receives its name because of its occurrence in shark liver oil (*Squalus* species) which contains large quantities and considered the richest source of squalene. Squalene is a natural antioxidant, a unique oxygen generator, power immune stimulator, antibiotic, anti-coagulant, anti-histamine and anti-allergic (Kelly, 1999). It has been proposed to be an important part of the Mediterranean diet as it may be a chemo preventative substance that protects people from cancer (Smith and Theresa, 2000; Owen *et al.*, 2004).

Thus each compound identified in leaf extract of *Ocimum americanum* has its own biological importance and further study of phytochemicals present in this plant can prove its medicinal importance in future and can be an effective and efficient drug source in cheaper rate as it has better availability.

3. 1. GCMS Profile of leaves of *O. americanum*

The spectra of GCMS and analyzed results which include the active principles with their molecular formula are given as below-

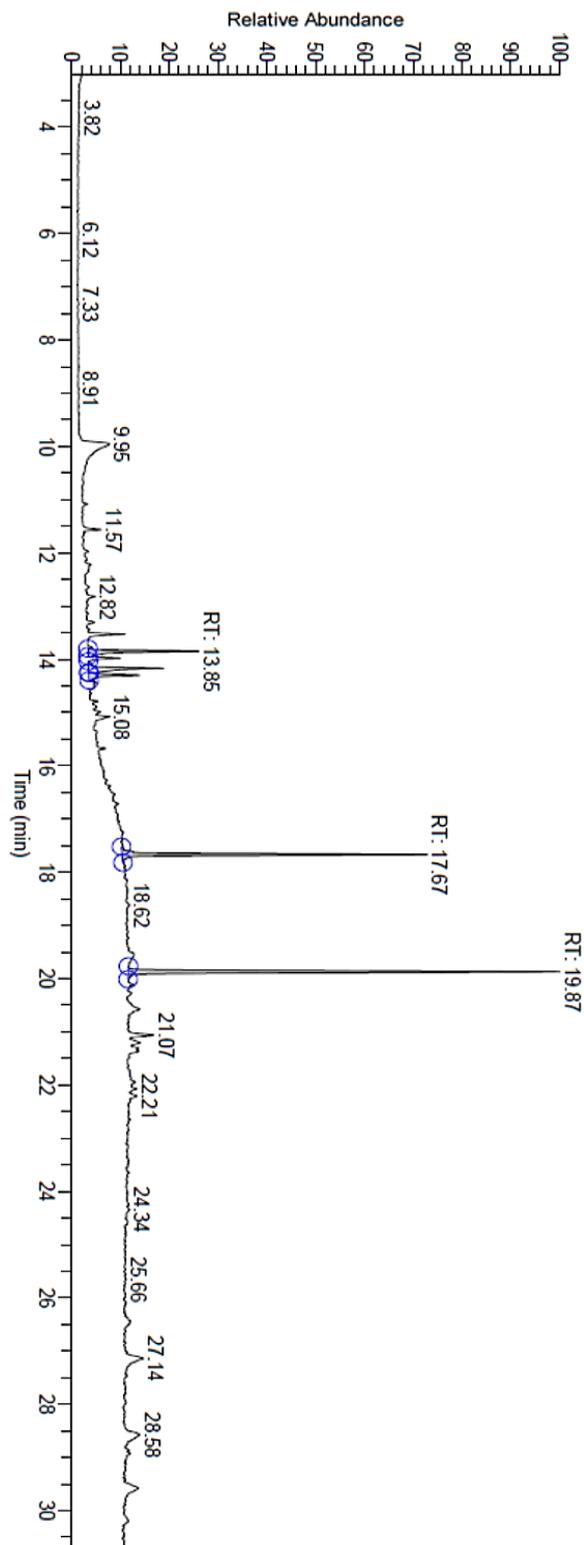


Figure 1. Spectra of Gas chromatography of Leaves of *Ocimum americanum* in methanol extract showing component peaks at different Retention time.

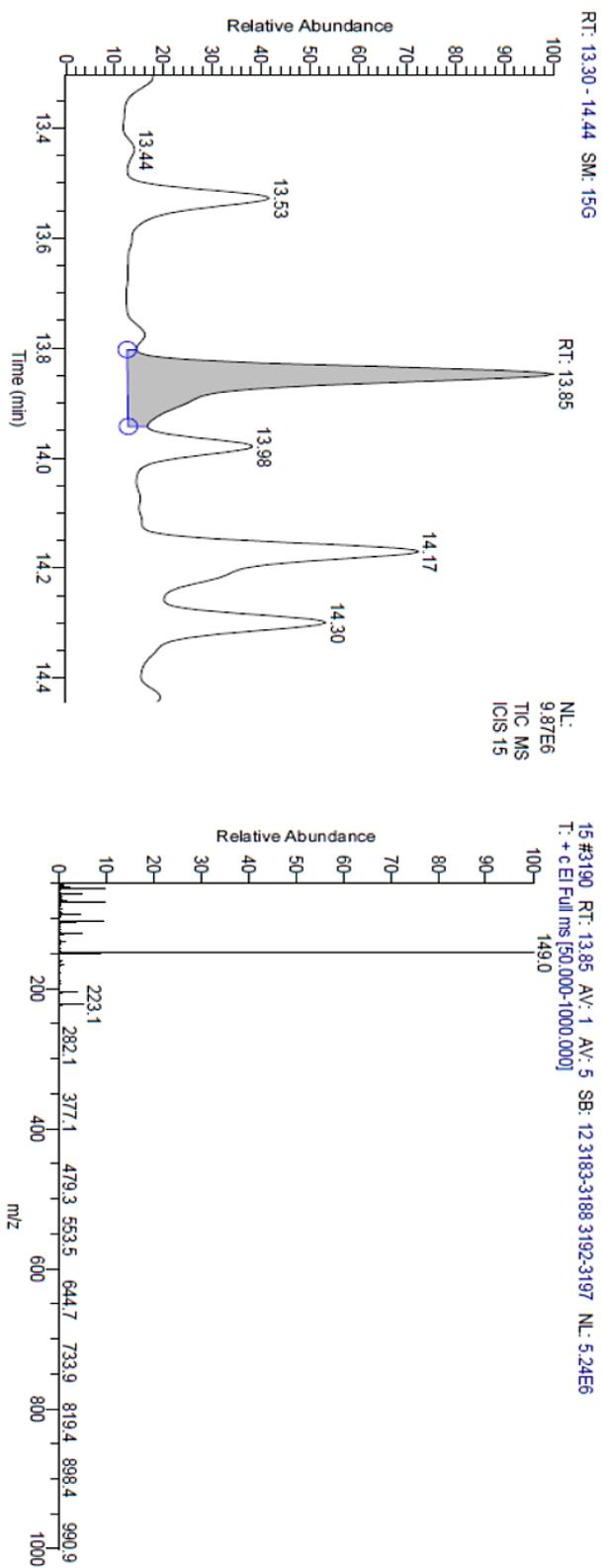


Figure 2. Peak fragmentation of GCMS at RT- 13.85

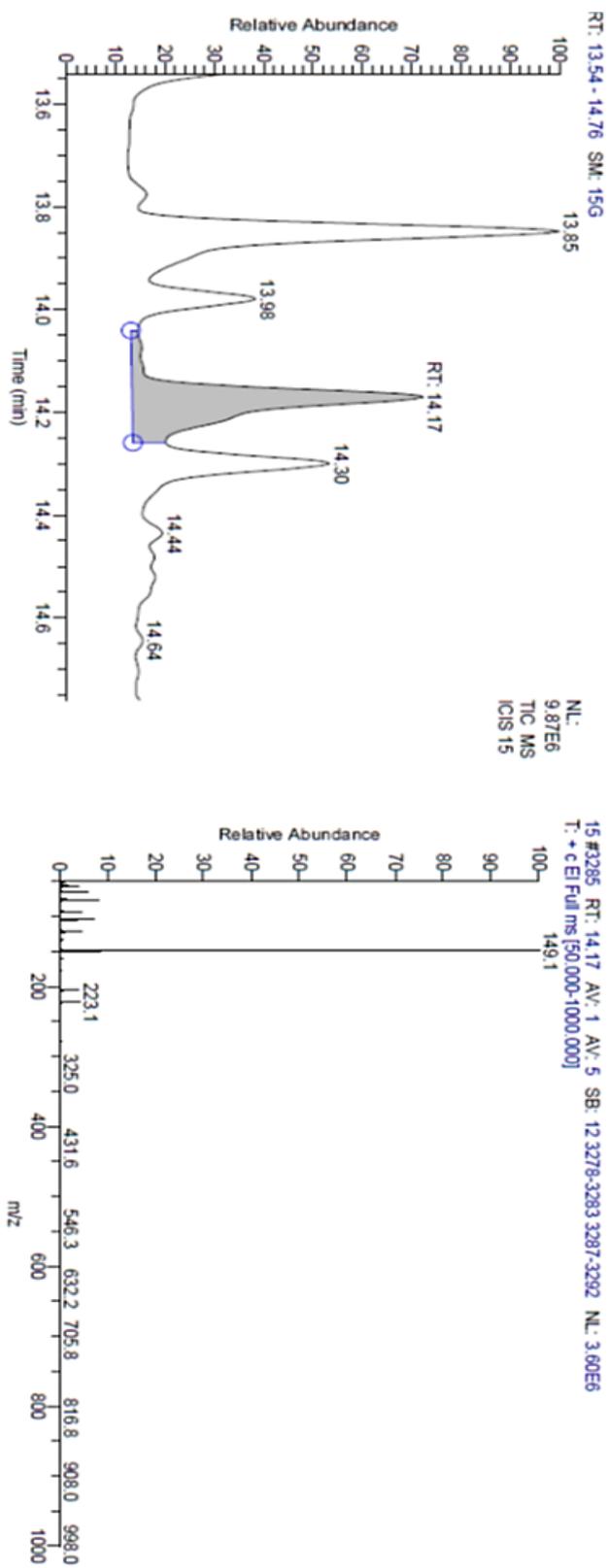


Figure 3. Peak fragmentation of GCMS at RT- 14.17

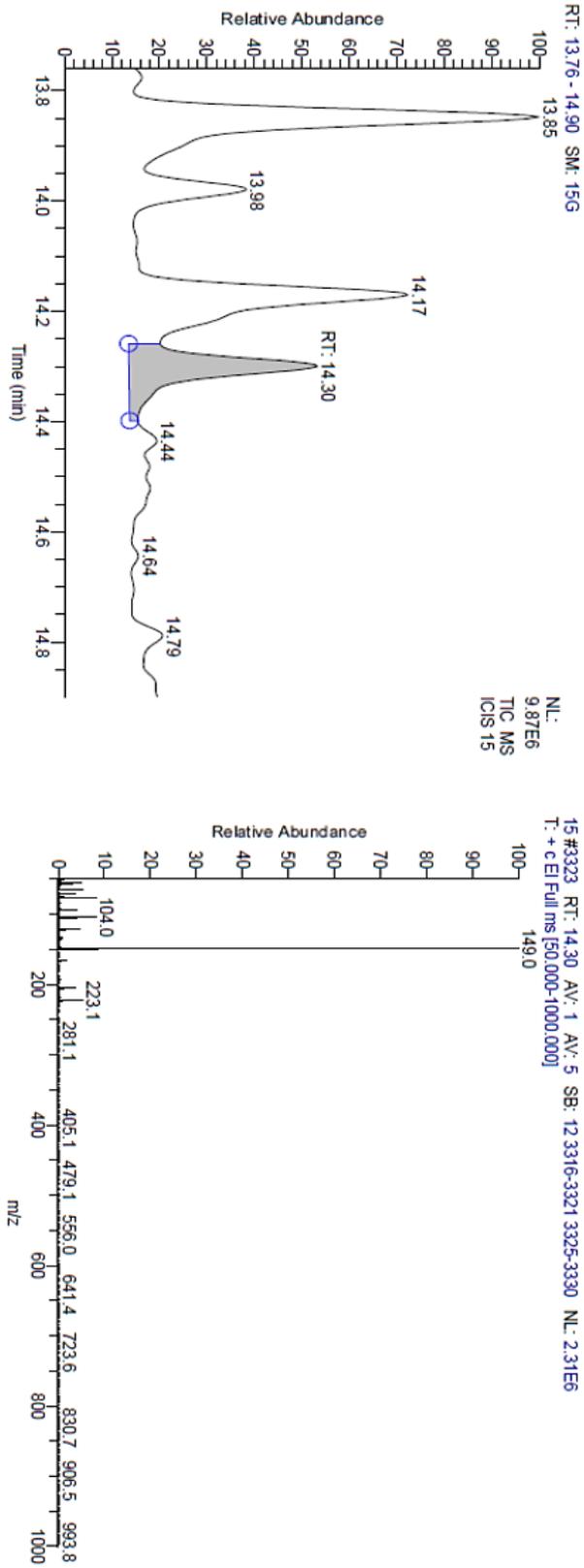


Figure 4. Peak fragmentation of GCMS at RT- 14.30

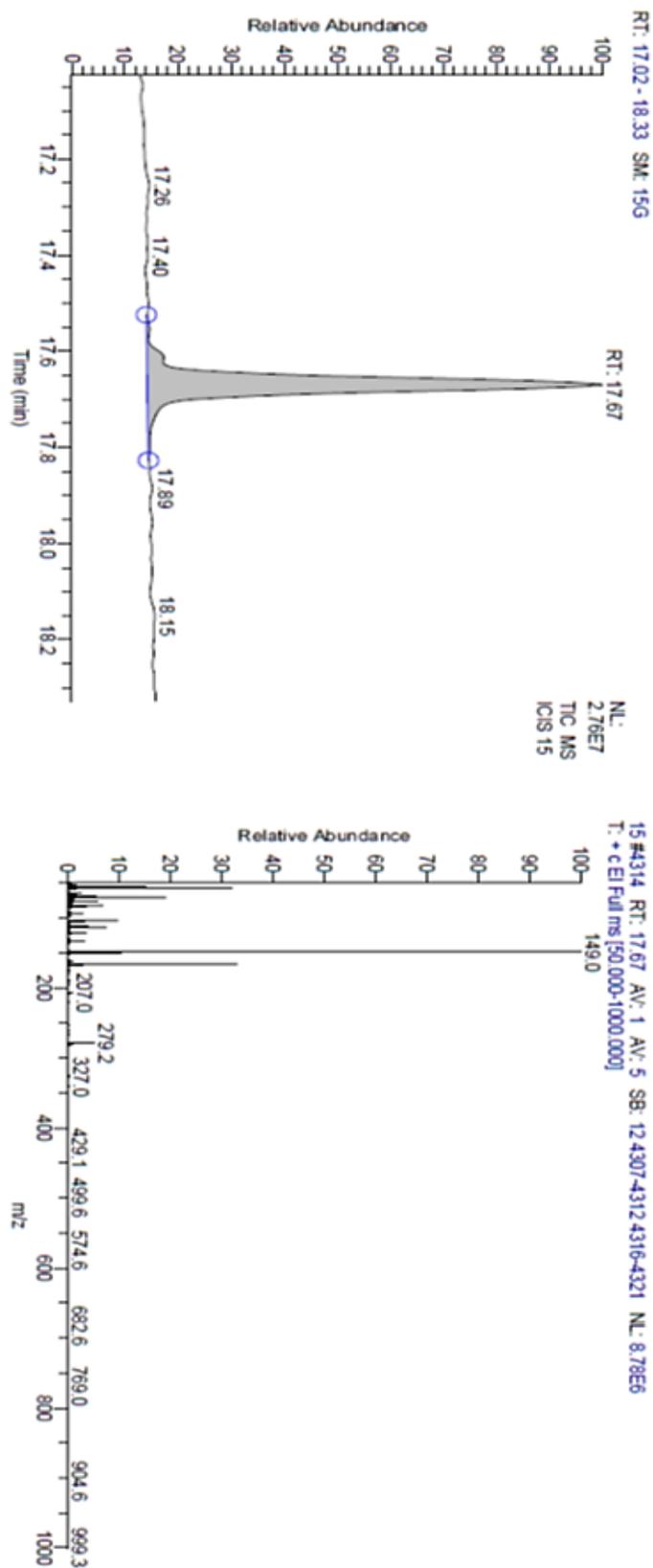


Figure 5. Peak fragmentation of GCMS at RT- 17.67

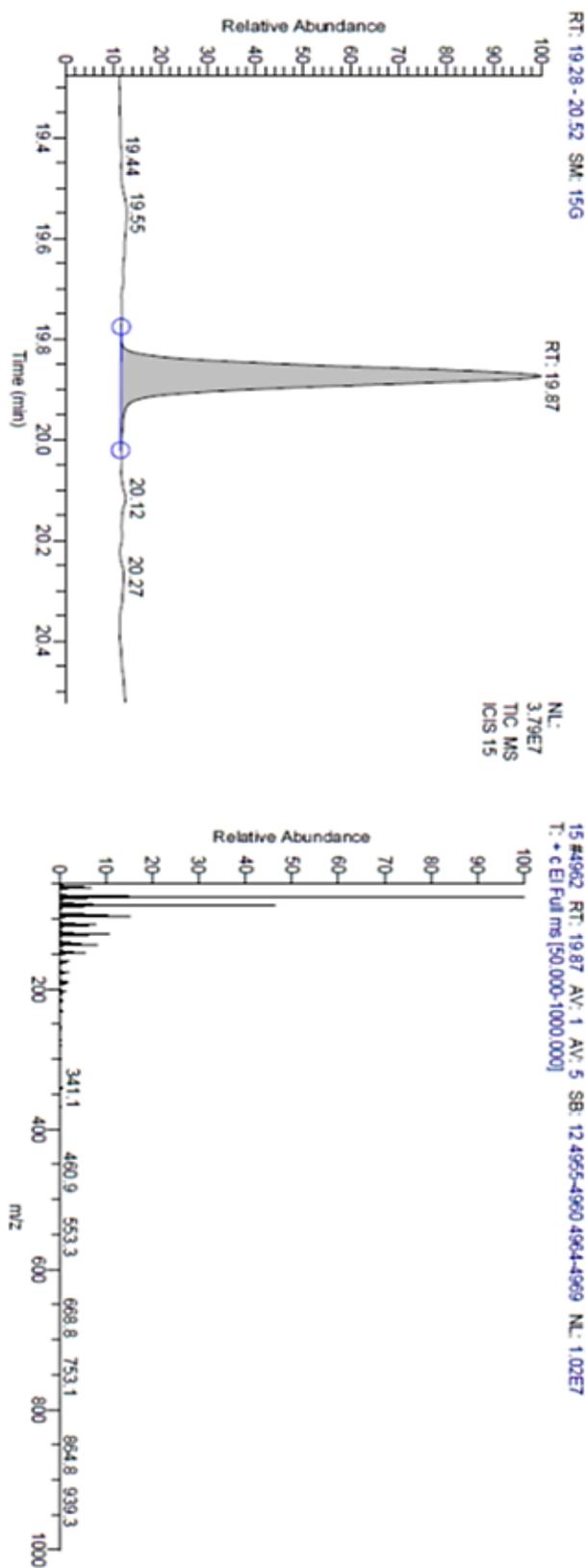


Figure 6. Peak fragmentation of GCMS at RT- 19.87

Table 1. List of expected Compounds at RT- 13.85

Sr. No.	Components	Retention Time (RT)	Expected Compound Name	Molecular Formula
1	1	13.85	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄
			1, 2- Benzenedicarboxylic acid	C ₁₆ H ₂₂ O ₄
			butyl 2- methylpropyl ester	
			1, 2- Benzenedicarboxylic acid, bis (2- methyl propyl) ester	C ₁₆ H ₂₂ O ₄

Table 2. List of expected Compounds at RT- 14.17

Sr. No.	Components	Retention Time (RT)	Expected Compound Name	Molecular Formula
1	2	14.17	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄
			1, 2- Benzenedicarboxylic acid, butyl 2- ethyl hexyl ester	C ₂₀ H ₃₀ O ₄
			1, 2- Benzenedicarboxylic acid, butyl 2- methyl propyl ester	C ₁₆ H ₂₂ O ₄

Table 3. List of expected Compounds at RT- 14.30

Sr. No.	Components	Retention Time (RT)	Expected Compound Name	Molecular Formula
1	3	14.30	Phthalic acid, butyl 2- pentyl ester	C ₁₇ H ₂₄ O ₄
			Phthalic acid, butyl hept-4-yl ester	C ₁₉ H ₂₈ O ₄
			Phthalic acid, hex-3-yl ester	C ₁₈ H ₂₆ O ₄

Table 4. List of expected Compounds at RT- 17.67

Sr. No.	Components	Retention Time (RT)	Expected Compound Name	Molecular Formula
1	4	17.67	Phthalic acid, Di (2- propyl pentyl) ester	C ₂₄ H ₃₈ O ₄
			Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄

Bis (2-ethyl hexyl) phthalate

$C_{24}H_{38}O_4$

Table 5. List of expected Compounds at RT- 19.87

Sr. No.	Components	Retention Time (RT)	Expected Compound Name	Molecular Formula
1	5	19.87	Squalene Trans geranylgeraniol	$C_{30}H_{50}$ $C_{20}H_{34}O$

Table 6. List of expected compounds and their activity.

Sr. No.	Expected Compound Name	Molecular Formula	Activity
1	Dibutyl phthalate	$C_{16}H_{22}O_4$	Antimicrobial and Antifouling
2	Phthalic acid	$C_{18}H_{26}O_4$	Antimicrobial
3	1, 2-Benzenedicarboxylic acid	$C_{16}H_{22}O_4$	Antimicrobial and Antifouling
4	Bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$	---
5	Diisooctyl phthalate	$C_{24}H_{38}O_4$	Antimicrobial and Antifouling
6	Squalene	$C_{30}H_{50}$	Chemo preventive
7	Trans-Geranylgeraniol	$C_{20}H_{34}O$	---



Figure 7. External view of *Ocimum americanum*.

4. CONCLUSION

The fundamental reason of quality control of herbal medicines is based on the concept of phytoequivalence of herbs, and then to use this conception to identify the real herbal medicine and the false one, and further to do quality control. Therefore, GC-MS method is a direct and fast analytical approach for identification of phytoconstituents. The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of components in *Ocimum americanum* suggests that the contribution of these compounds on the pharmacological activity should be evaluated.

References

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