www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.1, pp 250-257, Jan-Mar 2012

Volatile Oil of *Mimusops elengi Linn* (Sapotaceae) as a Source of Thymol

Sushil S. Pimpare*, Chetan A. Chaudhari, Lalit P. Sali,

Naveenkumar P. Jain, and **Chhaya H. Gadgoli***

Department of Pharmacognosy, Saraswathi Vidya Bhavan's College of Pharmacy, Sonarpada, Kalyan Shil road, Dombivli (E) - 421204, District-Thane, Maharashtra, India.

Corres.author: sushil.pimpare@gmail.com
Fax no.: 0222871243

Abstract : *Mimusops elengi*, commonly called 'Bakul' is a medicinally important plant of family sapotaceae. All parts of the tree have medicinal properties. Taking into consideration the medicinal importance of the plant, the volatile organic matter from the bark of this plant was analyzed for the first time for Thymol using GC-MS and HPTLC analysis.
Key Words: *Mimusops elengi*, *Sapotaceae*, GC-MS, HPTLC, Thymol.

INTRODUCTION

Mimusops elengi, commonly called as 'Bakul', is a medicinal plant belonging to family Sapotaceae. It is a small to large evergreen tree up to 15 m in height. All parts of the tree have medicinal properties. The bark, flowers and fruits are acrid, astringent, cooling and Anthelmintic (1). The bark is used as a tonic (1-4), febrifuge, as a gargle for odontopathy, inflammation and bleeding of gums (1). Powder of dried flower is a brain tonic and is useful as snuff to relieve cephalalgia (1). Young twigs are used for cleaning teeth (2). It is antipyretic and increases fertility in women (1,3). It is also useful in urethrorrhoea, cystorrhoea, diarrhea and dysentery. Flowers are used for preparing lotion for wounds and ulcers (3). Unripe fruit is used as masticatory and helps to fix loose teeth. Seeds are used for preparing suppositories in cases of constipation especially in children (2-4). Ripe fruit pulp is useful in chronic dysentery (3,4). Leaves are used in snake bite (3,4). With reference to the above facts, the bark has been examined to know the constituent of volatile organic matter. One of the ways by which essential oils or the volatile organic matter is extracted from plant material is through steam distillation (5).

In the present study, volatile organic matter of the bark sample of plant was analyzed for the first time for Thymol. This work will help to identify the compounds, which may be used in body products or of therapeutic value. GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc.

EXPERIMENTAL

The plant material used in this study was collected from the Mumbai market. It was authenticated at Agharkar Research Institute and the voucher specimen(S/B – 086) is deposited in the institute. The air dried bark (50 g) was soaked in distilled water (500 ml) and the volatile oil from the bark was obtained by distillation process. The distillate obtained was then extracted with solvent ether to separate the volatile organic matter and this was analyzed using GC-MS to detect and identify various constituents .

GC-MS analysis

The composition of the oil was analyzed using a GC-MS instrument (GCD - HP1800A). The oil sample was submitted to IIT, Mumbai to record GC-MS spectra. The GC-MS system was equipped with capillary column of HP-1 (30 m x 0.25 mm; film thickness 0.25 µm) and a split injector (split ratio 1:100). The oven temperature was programmed from 100ºC to 250ºC at the rate of 10ºC/minute and held at this temperature for 3 minutes and further increased to 280ºC at 30ºC/minute and held at this temperature for 3 minutes. The injector and detector temperature were set at 250ºC and 280ºC, respectively. 0.1 µl of oil was injected into GC-MS instrument for analysis. Helium gas was used as carrier gas at flow rate of 1 ml/min. The quadrapole mass analyzer contained an electron impact ion source with filament potential of 70 eV. The chemical components of the essential oil were identified by comparing their mass fragmentation patterns with those on the stored NIST library (National Institute of Standards and Technology) and Terpenoid library. Their structures were defined by the % similarity values.

Further for confirmation of presence of Thymol GC-MS analysis was performed on GC-MS instrument (GCD - HP1800A) system equipped with capillary column of HP-5 (30 m x 0.25 mm; film thickness 0.25 µm) and a split injector (split ratio 1:100). The oven temperature was programmed from 100ºC to 150ºC at the rate of 5ºC/minute and further increased to 250ºC at 10ºC/minute and further increased to 280ºC at 30ºC/minute. 0.1 µl of oil was injected into GC-MS instrument for analysis. Helium gas was used as carrier gas at flow rate of 1 ml/min. The quadrapole mass analyzer contained an electron impact ion source with filament potential of 70 eV.

HPTLC analysis

The HPTLC fingerprinting of the volatile oil was carried out for confirmation of Thymol using the reference standard (Loba Chemie). The volatile oil was dissolved in chloroform to obtain approx, 2 %w/v solution. The solution (200µl) was applied on HPTLC (Silica gel 60 GF_{254}; Merck, Germany) plates using Camag Linomat V Applicator fitted with a 100 µl Hamilton syringe. After drying of the spots, the plates were developed to a distance of 80 mm in a Camag twin-trough chamber previously saturated with mobile-phase vapor for 20 min.The mobile phase toluene : ethyl acetate (93 : 7) was used for Thymol detection (6). Densitometric evaluation of the plates was performed at λ = 254 nm, using a Dueterium lamp, with a Camag Scanner III in conjunction with winCATS Planar Chromatography Manager software, version 1.4.4.6337. After development, the plates were derivatized with freshly prepared Anisaldehyde sulphuric acid reagent in a derivatization chamber for 20 seconds and dried at room temperature. After drying, plates were heated in an oven at 105ºC for 10 minutes for the photograph. Presence of Thymol is also proved by comparison of reflectance UV spectra of the standard and the sample peak.

RESULTS AND DISCUSSION

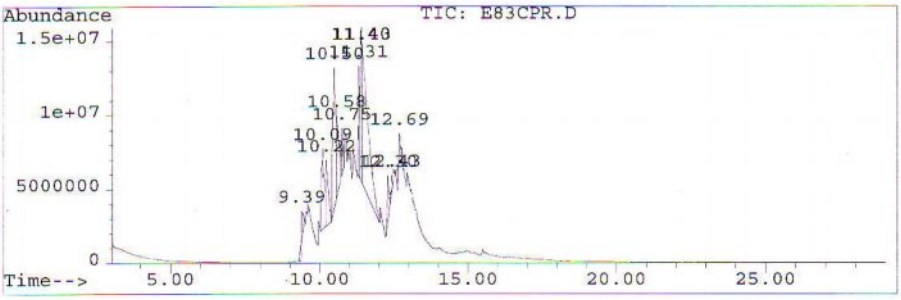
Volatile organic materials are products of the secondary metabolism of plants, and are generally consisting of complex mixtures of mono-, sesqui-, di-, hydrocarbons, and oxygenated materials biogenically derived from them.

Water distillation of bark sample yielded 0.18% w/w of volatile organic matter. Use of GC-MS enabled identification of chemical constituents present in it. The GC-MS analysis of volatile oil of bark of *M. elengi* as seen in (Figure 1,2).indicate the presence of 12 compounds. The MS analysis of the eight compounds could be matched with NIST library (Fig.4.21). Their structures are proposed based on the % similarity values. Some of the compounds identified are listed in Table 1.

Figure 1 Area Percent Report of the constituents of the volatile oil

Area Percent Report -- Sorted by Signal

Information from Data File:
File : C:\HPCHEM\1\E83CPR.D
Operator : SHARLET
Acquired : 19 Mar 110 1:50 pm using AcqMethod MP
Sample Name: SAMPLE-3
Misc Info : 100-3M-10-250-3M-30-280-8M-HP1-30m-0.25 ID
Vial Number: 1
CurrentMeth: C:\HPCHEM\1\METHODS\MP.M



Retention Time	Area	Area %	Ratio %	Type	Width
Total Ion Chromatogram					
9.390	47490254	1.292	3.662	BB	0.036
10.095	375327877	10.209	28.946	PV	0.098
10.224	257671935	7.009	19.872	VV	0.075
10.495	635178774	17.277	48.985	PV	0.089
10.577	280953610	7.642	21.667	VV	0.085
10.751	83314514	2.266	6.425	VV	0.039
11.314	202850276	5.518	15.644	BV	0.043
11.398	365343663	9.937	28.176	VV	0.060
11.434	1296669360	35.270	100.000	VV	0.230
12.299	103049747	2.803	7.947	PV	0.052
12.431	26529123	0.722	2.046	VV	0.044
12.686	2070039	0.056	0.160	BV	0.016

C:\HPCHEM\1\E83CPR.D Mon Mar 22 10:00:45 2010 Page 1

Figure 2 Gas chromatogram of the volatile oil of the bark of *M. elengi*

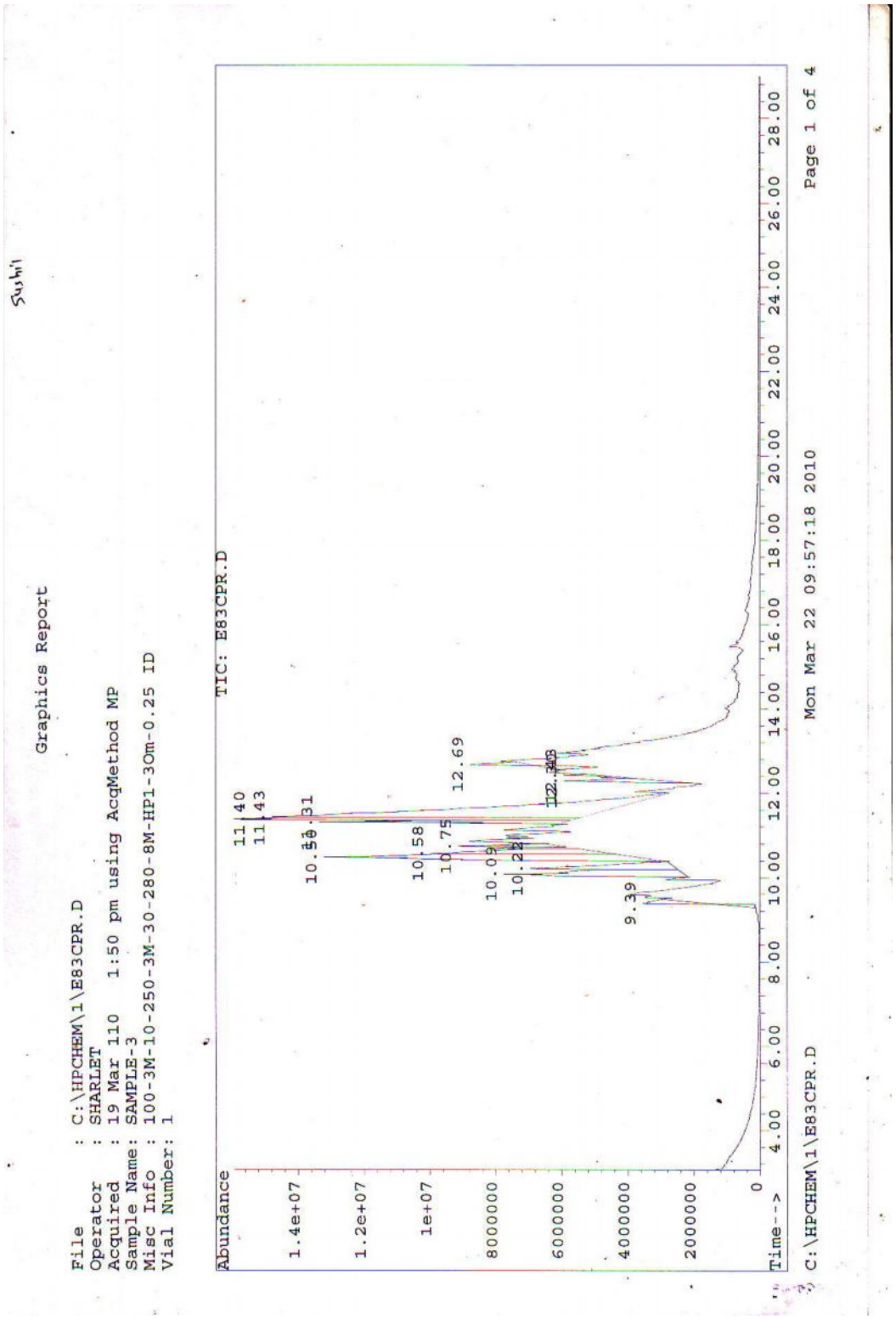
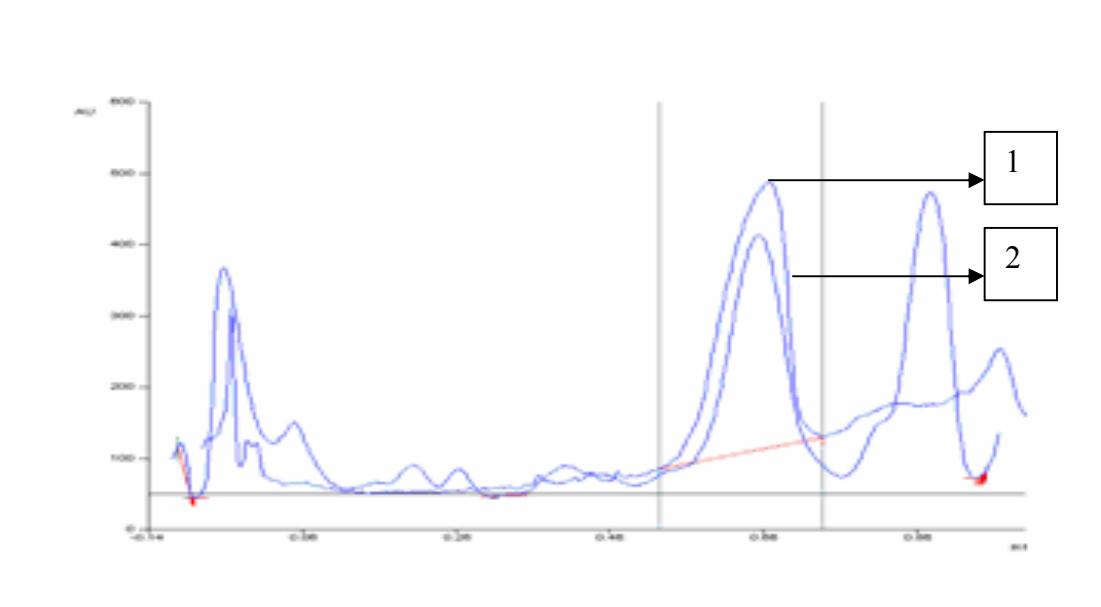


Table 1 GC-MS analysis of the volatile oil

Sr.No	Retention Time	Area %	Name of Compound	% Similarity	Molecular ion peak
1	9.39	1.29	Linalol	91%	155
2	10.09	10.21	Copaene	99%	204
3	10.49	17.28	Isosafrol	95%	162
4	10.58	7.64	β -caryophyllin	89%	204
5	10.75	2.27	Safrol	96%	162
6	11.32	5.52	δ-cadinene	97%	204
7	11.40	9.94	Phenol,2,5-bis(1-methylethyl)-(Thymol)	38%	178
8	12.43	0.72	γ-cadinene	51%	204

Figure 3 Chromatograms of Standard Thymol and volatile oil of bark of *M. elengi* at 254nm

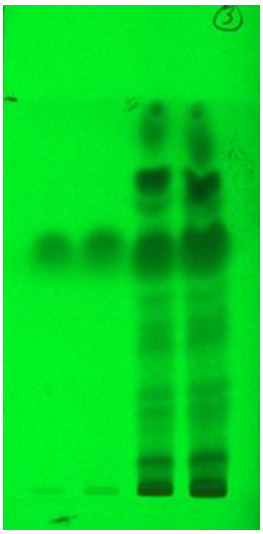


Note:
1 Chromatogram of Standard Thymol (R_f 0.52)
2 Chromatogram of volatile oil of bark of *M. elengi* (R_f 0.52)

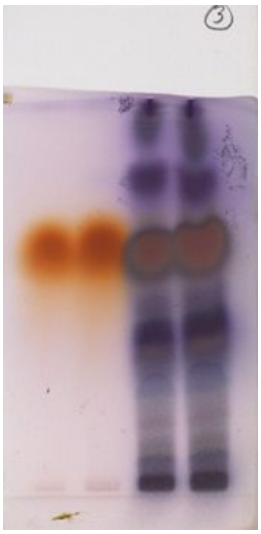
Table 2 Chromatographic data of HPTLC analysis of standard Thymol and volatile oil (at 254 nm)

Peak	Start Position (R_f)	Start Height (AU)	Max Position (R_f)	Max Height (AU)	Max %	End Position (R_f)	End Height (AU)	Area (AU)	Area %	Assigned substance
1	0.52	4.6	0.64	355.6	100	0.70	0.8	31416.2	100	Thymol
2	0.52	2.7	0.62	389.2	100	0.70	2.6	25372.0	100	Methanolic extract

Figure 4 HPTLC Plate image of volatile oil

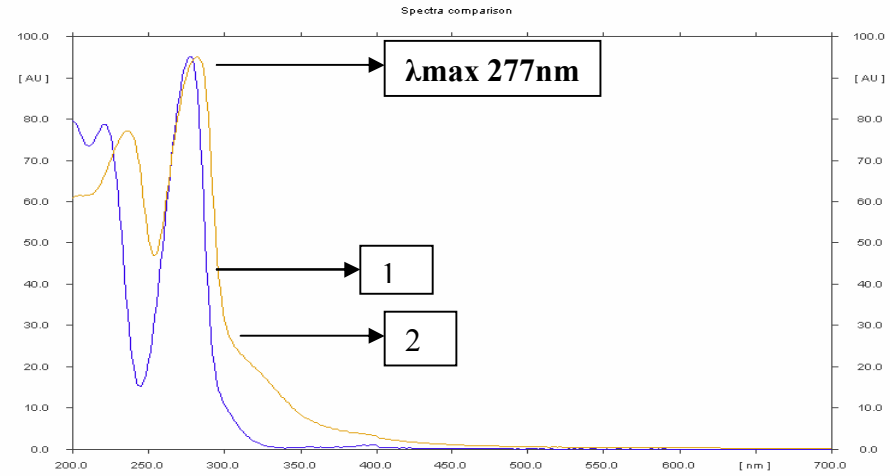


A. Visualization at 254 nm



B. derivatized with Anisaldehyde sulphuric acid reagent

Figure 5 Superimposition of the UV spectra of Thymol reference standard and spot of Thymol from volatile oil of bark of *M. elengi Linn.* (λmax 277nm)



1: UV spectrum of spot with R_f 0.52 from essential oil of bark of *M. elengi Linn.*
2: UV spectrum of Thymol reference standard

Figure 6 The Gas Chromatogram of the volatile oil of bark of *M. elengi Linn.*

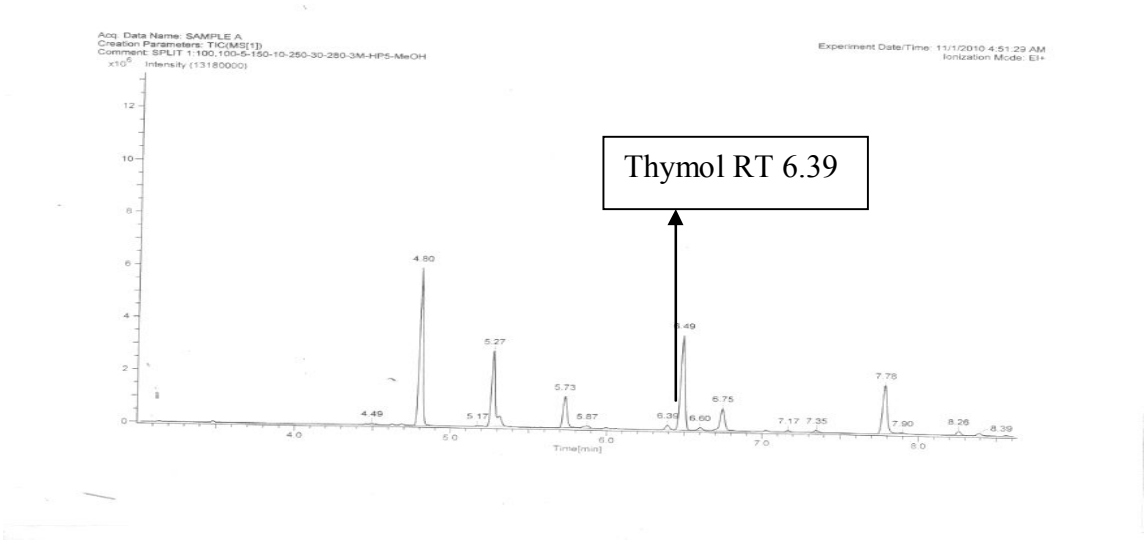


Figure 7 Mass spectra of the Thymol present in volatile oil (RT 6.39**) of bark of *M. elengi Linn***

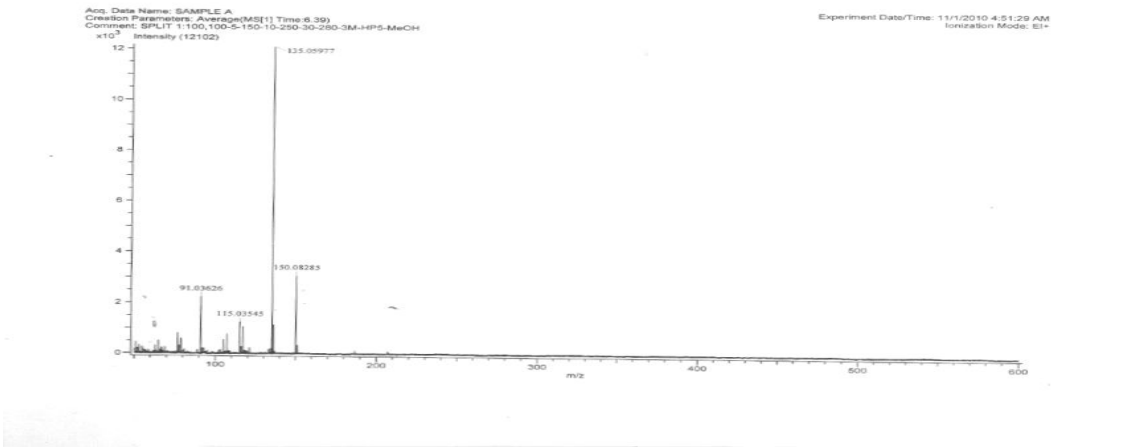


Figure 8 The Gas Chromatogram of the standard Thymol

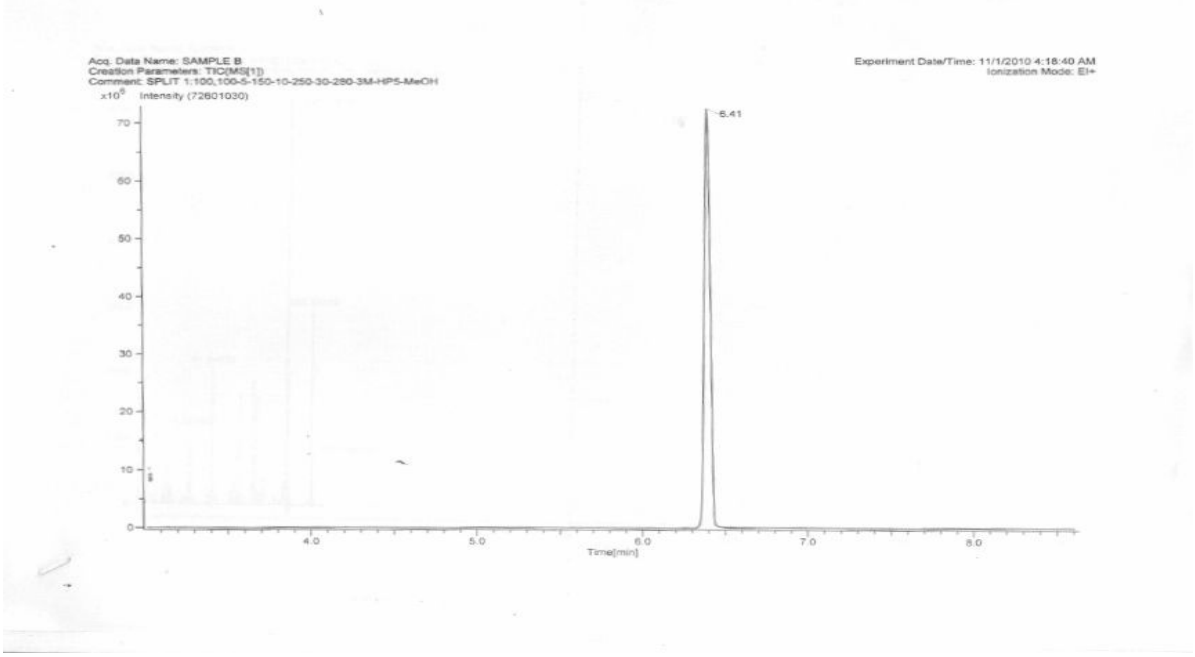
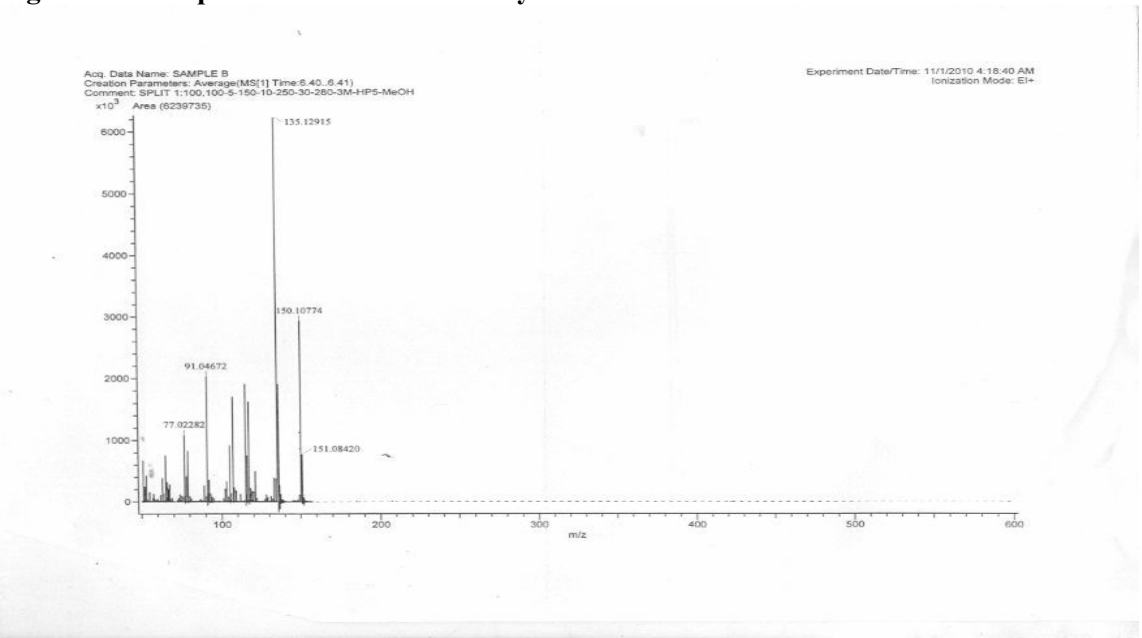


Figure 9 Mass spectra of the standard Thymol



The probabilities given for the component with retention time 11.40 and molecular weight 220 was Phenol,3,5-bis(1-methylethyl). Phenol,3,5-bis(1-methylethyl) may be Thymol or Thymol derivative. From the Qualitative HPTLC analysis it was observed that, the R_f of the components of the oil matched with standard Thymol (R_f 0.52) (Figure 3 and Table 2). Also the reaction of the component and Thymol with Anisaldehyde sulphuric acid reagent yielded brown colour (Figure 4). Presence of Thymol is also proved by comparison of reflectance UV spectra of the standard and the sample peak (λmax 277nm) (Figure 5).

Further GC-MS analysis was performed for Thymol and volatile oil which confirms the presence of Thymol in oil as retention time (RT 6.39) and mass spectra of oil component were exactly match with that of standard Thymol (Figure 6-9).

REFERENCES

1. Database on Medicinal Plants used in Ayurveda,1, Central Council for Research in Ayurveda and Siddha, Department of ISM & H, Ministry of Health and Family Welfare (Govt. of India), 2000, 65-68.
2. The Wealth of India, Raw Materials, CSIR, New Delhi, India, 1962, 6, 383-384,
3. Joshi S.G., Medicinal Plants, Oxford & IBH publishing Co. Pvt. Ltd, 2000, 362.
4. Chopra R.N., Nayar S. L and Chopra I.C., Glossary of Indian Medicinal Plants, National Institute of Science Communication and Information Resources (CSIR), New Delhi 2000, 167.
5. Clifford, A.A. "Extraction of Natural Products with superheated water" In Proceedings of the GVC-Fachausschss 'High Pressure Chemical Engineering', Karlsruhe, Germany, Mar 3-5 , 1999.
6. Wagner H. and Bladt S., Plant Drug Analysis- A Thin Layer Chromatography Atlas, Springer-Verlag Berlin Heidelberg, New York, 2nd Edition, 1996, 152-153.

*****