SUPERCRITICAL FLUID EXTRACTION AND CHARACTERIZATION OF ACTIVE COMPOUNDS FOR PHARMACEUTICAL IMPORTANCE FROM BOTANICALS OF EUPHORBIACEAE FAMILY

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ABSTRACT

Euphorbia thymifolia belongs to Euphorbiaceae family, are known as weed. The extracts obtained by SCFE technique and solvent extraction methods have been evaluated for chemical components using GC-MS instrument. The chromatographic analysis showed that supercritical CO_2 extract contained more components than methanol extract. Tricosane hydroquinone, fridoline, 5-hydroxy-4 methoxy-7-methyl flavone and euphorbal have been identified.

INTRODUCTION

The plant, *Euphorbia thymifolia* L. belong to Euphorbiaceae family grown in subtropical areas in India as a weed. The whole plant is used as a medicine in Indian traditional system of medicine since ancient time as antibacterial, antidysenteric and vermifuge. It is a small annual herb with opposite elliptic leaves 4-8 mm long 2.5 mm wide, delicate roots, having milky latex in stem, which spread on the ground as spurge[1,2]. It is considered as stimulant, astringent, anthelmintic and laxative given to children in bowl complaints. The essential oils obtained from the plants are used in medicinal soaps for the treatment of erysipelas as a spray to keep off flies and mosquitoes[3,4,5,6].

MATERIAL AND METHODS

The whole plant is collected from herbal garden (Micro model) at Indian Institute of Technology, New Delhi. The plant is soaked in water to remove mud, foreign matters etc. and dried in shade. The shade dried material was cut into pieces and ground to a fine powder. For supercritical extraction process, air dried leaves of these plants were crushed to powder which were then sieved and particle size of 0.5 mm was packed in extractor thimble using glass wool on both the ends. The extractor was then connected to the rest of assembly and carbon dioxide was passed through the sample present in extractor thimble, desired sub/ super critical temperature was maintained with the help of water thermostat and air-driven pump was used to push carbon dioxide to maintain pressures (e.g. 80, 100, 120 and 150). The extract from sample was collected, weighed before and after the experiment and the yield was obtained by subtracting the two weights[7,8,9,10].

METHANOL SOLVENT EXTRACTION

The plant powder 30 gm in a paper thimble was taken and extracted in methanol solvent using soxhlet apparatus around 8-10 hours[8]. The solvent is evaporated under reduced pressure. The extracts obtained by both the methods were put for GC-MS and results were compared[9].

GAS CHROMATOGRAPHIC-MASS SPECTROMETRY ANALYSIS

The extract obtained by SCFE is dissolved in diethyl ether and soxhlet extract is redissolved in methanol and the samples of 0.01% for analysis were prepared.

The GC-MS analysis was performed using a Hewlett-Packard HP 6890 series chromatograph interfaced with a HP 5973 mass selective detector and with HP 7683 series injector. The gas chromatography column was a HP-5MS 5% phenylmethyl siloxane capillary column (30.0m x 250 μm x 0.25 μm). Helium was used as carrier gas with a flow rate of 0.7 ml/min and temperature programming employed during the analysis. An injector temperature of 290°C was used, and the initial oven temperature of 50°C while injection of each sample (1.0 μl). Thereafter a heating rate of 2°C/min was applied until a temperature of 200°C was reached and it was maintained for a period of 10 minutes, at which time the heating rate was increased to 5°C/min to increase the temperature at 290°C, which was then maintained for 10 minutes.

The mass spectrometer was operated in the EI mode and mass spectra were obtained at 70eV, 100μA using a source temperature of 220°C and a pressure of approximately 2×10^6 Torr throughout the analysis.

Compounds were identified by their characteristic mass spectrometric fragmentation patterns by direct automatic comparison with library spectra, the instrument used has direct access to NIST and WILEY spectral libraries[10].

RESULT AND DISCUSSION

The physico chemical analysis of air-dried crushed powder of *E. thymifolia* yields the loss on drying 68.31%, Ash content 9.10%, Acid insoluble ash volume 1.59%, supercritical CO_2 extraction in 70 minutes at 120 bar and 25^{o}C, 2.90% w/w basis. Solvent methanol extract in 8 hours gives the yield 3.03%[11, 12]. The SCFE process was found superior and faster to organic solvent extraction methods. The conventional process requires more time to separate out the chemical components from the plant matrix, hence consume more energy in case of hexane extraction. It was also observed in SCFE extraction, the plant yields a green colour liquid with a peculiar pungent odour and irritating taste. The detailed study for the chemical components present in the extract is given in subsequent tables and figures. The extraction in supercritical CO_2, in methanol and hexane solvents from *E. thymifolia* L. were analysed by thin layer chromatography (TLC) and gas chromatographic-mass spectrometry analysis.

THIN LAYER CHROMATOGRAPHY (TLC)

The SCFE extract was dissolved in a known volume of the methanol to make 0.01 concentrations. The TLC plate was developed in the toluene : ethylacetate : diethylanine (70:20:10) solvent system. The four spots were analysed at the R_f values of 0.04 (red), 0.85 (light green), 0.88 (pale yellow) and 0.94 (red). The same sample was applied in the TLC plate and developed in EtOH : MeOH : EtAct : H_2O) (81:11:4:8) solvent system. The three spots were analysed at the R_f values 0.65 (light green), 0.80 (orange) and 0.94 (red) [11, 12].

GC-MS ANALYSIS

In general, the supercritical fluid (CO_2) extract and solvent extract were found quite similar. In case of SCFE extract, 120 compounds were identified while in solvent extraction process, the 33 and 62 compounds were identified in methanol and hexane extracts respectively[10].

The important components identified in these extract are listed in Table 1, 2 and 3 presented in figure 1, 2 and 3.

Table 1: Important compounds identified in SCFE extract of *E. thymifolia* by GC-MS

Compound Name MF	**RT**	**Area %**
Hydroquinone $C_6H_6O_2$	27.83	1.87
2-phenyl-5-carboxy-thiophene	39.64	0.57
Hexadecane $C_{16}H_{34}$	46.84	0.47
4,5,6-Trimethoxyindole $C_{11}H_{13}NO_3$	48.44	0.48
Neophytadiene	61.05	0.89
Palmitic acid $C_{16}H_{32}O_2$	67.61	0.45
25,28-Dihydroxy-D:A-friedooleanan $C_{30}H_{50}O_3$	68.78	0.13
Androstan-3-ol $C_{19}H_{32}O$	74.67	0.67
Bicyclo-nonan-2-ol $C_9H_{16}O$	75.79	0.23
Methyl 3-(l-cyano-2-oxycyclooctyl) $C_{13}H_{19}NO_{13}$	75.83	0.18
5,10-Pentadecadienal $C_{15}H_{26}O$	77.09	1.47
N,N-Dimethylstearamide	80.91	0.62
Tricosane	114.95	0.85
Stigmast-22-en-3-ol, 4-methyl	116.31	0.24
Stigmasta-4,6,22-trien-3beta-ol $C_{29}H_{46}O$	125.61	0.25
Lanost-8-en-7-one $C_{30}H_{50}O$	125.83	0.32
Fern-9(11)-en-6-one $C_{30}H_{48}O$	130.21	4.29
6,7-Dimethoxyisoquinoline-l-carboxaldehyde $C_{12}H_{11}O_3$	132.28	2.29
D:C-friedo-oleana-7,9(11)-diene-3 $C_{30}H_{48}O$	132.57	5.01
D:B-Friedooleanan-3-ol, 5,10-epoxy-, (3ß,5ß,10ß)- $C_{30}H_{50}O_2$	132.65	3.59

Table 2: Important compounds identified in solvent (methanol) extract of *E. thymifolia* by GC-MS

Compound name MF	**RT**	**Area %**
Pyrimidine	3.29	1.06
4,7-Dimethyl-8-methoxycoumarin	36.53	2.91
2-Cydohexan-1-ol	46.10	4.97
9-benzyliden-4-azafluorene cis an	67.61	7.10
Isotirucallene	127.23	1.22
2,4,5,6,7-pentamethylisoindoline	132.08	6.21
11,14-diphenylpyridazine	136.21	1.33

Table 3: Compounds identified in solvent hexane extract of *E. thymifolia* by GC-MS

Compound name MF	**RT**	**Area %**
2-Ethyl-1-dodecanol	39.53	0.68
6-Ethoxy-1,2,3,4-tetrahydro-2,2,4-	39.91	1.12
Flavone, 5-hydroxy-4'-methoxy-7-methyl	77.09	0.38
Octadecane, 3-ethyl.5-(2-ethylbutyl)	114.87	1.19
Tricosane	114.90	1.14
1-phenyl-3,4-dihydroisoquinoline	127.53	0.30
Trans-2-(2-hydroxybenzyl) cyclohexone	132.06	2.68
24-Noroleana-4(23), 12-diene, 3-methyl	132.12	7.90

Table 4: Comparison of the compounds obtained in different extract of the *Euphorbia thymifolia.*

Compound name	SCFE Extract	MeOH extract	Hexane extract
Coumarin, 4,4,6,8-tetramethyl-	-ve	-ve	+ve
Dodecanoic acid	-ve	-ve	+ve
Flavone, 5-hydroxy-4’	-ve	-ve	+ve
Octadecane	-ve	-ve	+ve
Tricosane	+ve	-ve	+ve
Isoquinoline	+ve	-ve	+ve
Phenol	+ve	-ve	+ve
Isotirucallene	-ve	+ve	-ve
Cyclohexenol	-ve	+ve	-ve
Hydroquinone	+ve	-ve	-ve
Hexadecane	+ve	-ve	+ve
Euphorbol	+ve	-ve	-ve
24-Noroleana –4(23), 3-met	+ve	-ve	+ve

CONCLUSION

The *E. thymifolia* plant contains mainly 5,7,4 trihydroxy flavone-7-glycoside, tricosane, hydroquinone, fridoline and euphorbal. It also yields a green essential oils with its constituents, cymol, carbacrol, limonene, 2-sesquiterpenes have been identified. In general these compounds possess certain medicinal value. The compound 24–Novoleana-4(23), 12-diene 3 methyl was obtained at RT132. The compound tricosane is identified in both hexane and SCFE extract at 114.90 RT. Both these compounds were found in all the extracts of *E. thymifolia.* The intensity of peak and area of the peak for this Novoleana compound was large when compared to other compounds.

ACKNOWLEDGMENT

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REFERENCES:

[1] The Wealth of India, Vol. 3, **1989**, p. 225.
[2] FREISE, F. W., Perfumery and Essential Oil Record, Vol. 26, **1935,** p. 219-220.
[3] KHAN, N.H, *et al.*, Indian J Med Res., Vol. 87, **1988**, p. 395-397.
[4] RAO, V.R, GUPTA, I., Indian J Pharmacol, Vol. 27, **1970**.
[5] CHUN-CHINGLIN *et al*., Journal of Biomedical Science, **2002**, p. 655-664.
[6] AMARAL, A.C.F., *et al*., Fitoterapia, Vol. 70, **1999**, p. 293-295.
[7] SINGH, R., and MAHESHWARI, R.C., Indian Perfumer, Vol. 40(1), **1997,** p. 26-29.
[8] Current Science, Vol. 49 (8), **1980**, p. 717.
[9] MAHESHWARI, R.C., and LENTZ, H., Fluid Phase Equillibria, Vol. 41, **1988**, p. 195-204.
[10] MAHESHWARI, R.C., and NAIK, S.N., Supercritical Fluid Extraction Technology for Natural Products, **1995**.
[11] Council of European Pharmacopoeia, 3rd edn., **1997**, p. 121-122.
[12] British Pharmacopoeia, **1983**, p. A 77-A 140.

Figure 1: GC/MS spectra of SCFE extract dissolved in Diethylether of *Euphorbia thymifolia* L.

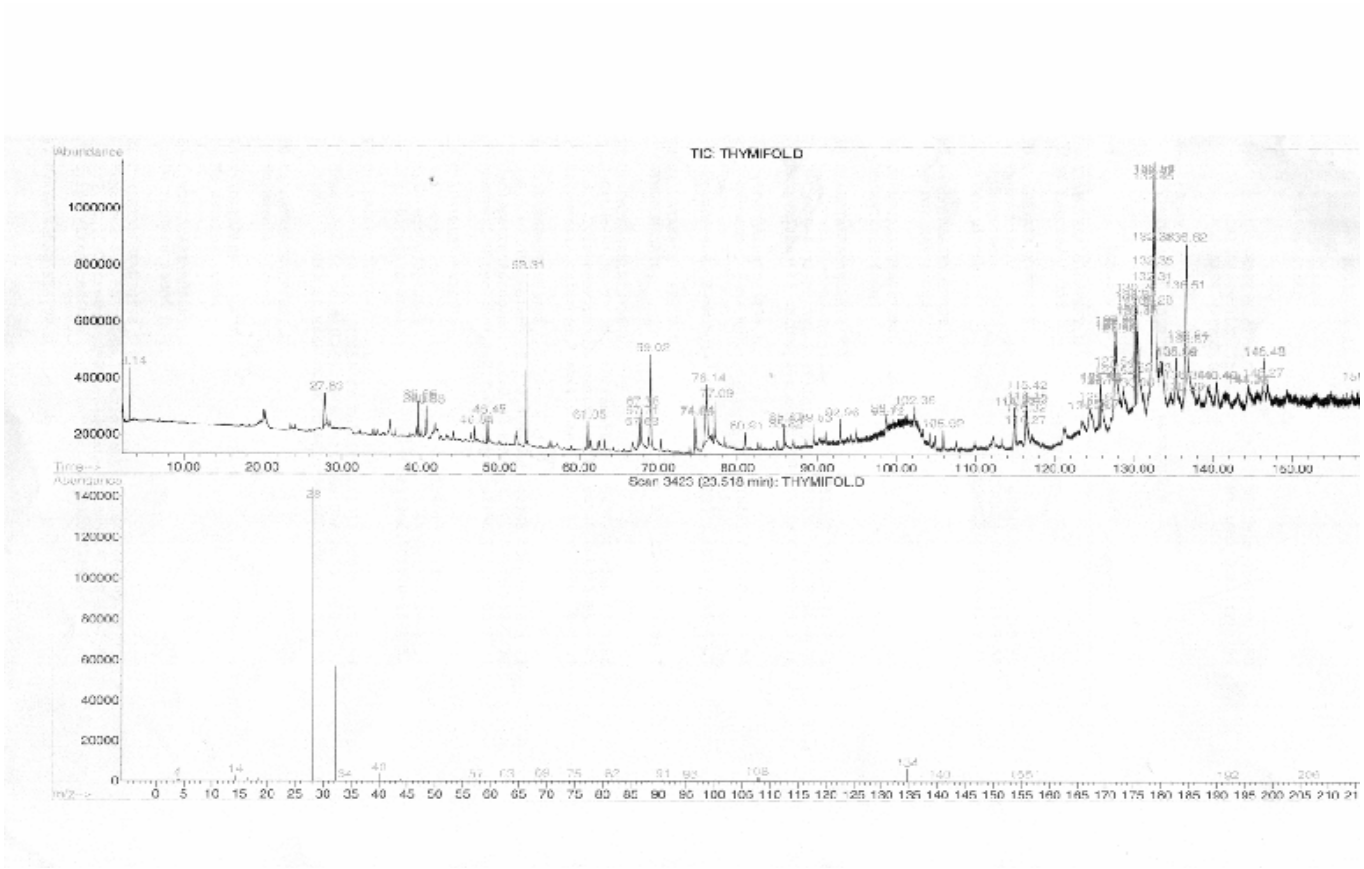

Figure 2: Figure 3.4. GC/MS spectra of Methanol Soxhlet extract of *Euphorbia thymifolia* L.

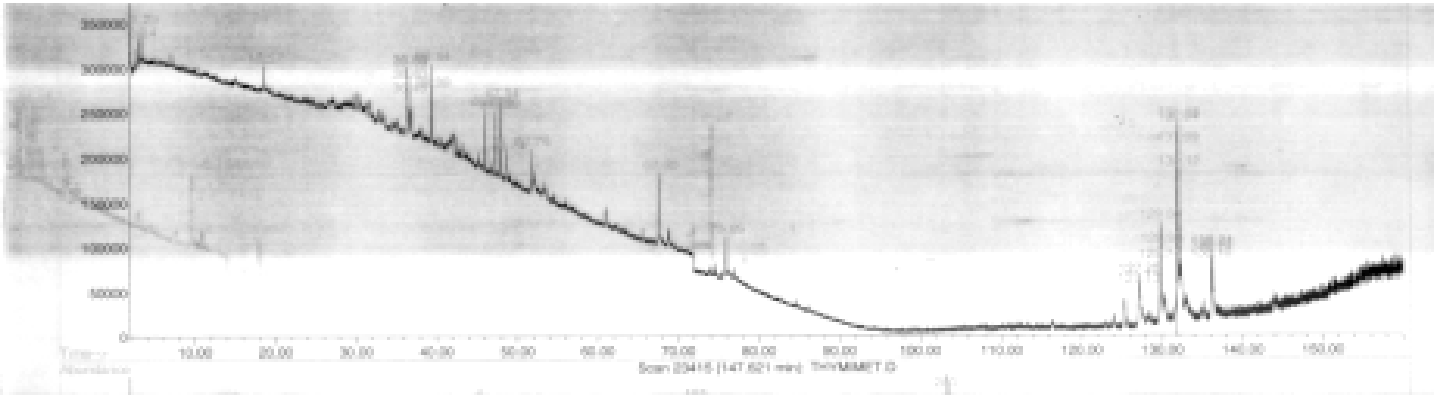

Figure 3: GC/MS spectra of Hexane extract of *Euphorbia thymifolia* L.