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Extraction and Characterization of Echinops Echinatus Plant Extract by Column Chromatography and GC-MS Analysis

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ABSTRACT

Echinops echinatus Family: Asteraceae is used as medicinal plant used in urinary disorder, liver disorder, heart diseases, etc. The seeds are sweet and aphrodisiac (Aurveda). The methanolic extract of leaves was obtained by Soxhelt extractor followed by concentration in rotary evaporator. Separation of bioactive chemicals was carried out by column chromatography while analysis by GC-MS which shows presence of following chemicals compositions. Phenol, 2,4bis(1,1dimethylethyl), Pentanoic acid, 5hydroxy,2,4ditbutylphenyl esters, 7,9Ditertbutyl1oxaspiro(4,5)deca6,9diene2,8dione, Benzo[b]dihydropyran, Propanoic acid, 2methyl,(dodecahydro 6hydroxy9methyl3methylene2,9dioxoazuleno[4,5b] furan6yl) methyl ester, Hexadecanoic acid,2hydroxy1(hydroxymethyl) ethyl ester, Glycerol 1palmitate, Octadecanoic acid, 2,3dihydroxypropyl ester, Octadecanoic acid,2hydroxy1(hydroxymethyl)ethyl ester, DISTEARIN, 13Docosenamide,(Z), Bis(cis13docosenamido) methane, Stigmasterol, 28,33 Dinorgorgost5en24one,3hydroxy, 3Dodecene,

Keywords: Column chromatography, GC-MS Analysis

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INTRODUCTION

Echinops echinatus Family: Asteraceae that is native to More or less throughout India and Afghanistan. It is an erect branched herb about a meter high. It has short, stout stems, branching from the base, covered with white cottony hair. It is used as medicinal plant used in urinary disorder, liver disorder, heart diseases, etc. The root is abortifacient aphrodisiac. The seeds are sweet and aphrodisiac (Ayurveda). [1]

Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids. This is a solid - liquid technique in which the stationary phase is a solid & mobile phase is a liquid. The principle of column chromatography is based on differential adsorption of substance by the adsorbent. The usual adsorbents employed in column chromatography are silica, alumina, calcium carbonate, calcium phosphate, magnesia, starch, etc., selection of solvent is based on the nature of both the solvent and the adsorbent. The rate at which the components of a mixture are separated depends on the activity of the adsorbent and polarity of the solvent.[2]

MATERIALS AND METHOD

Collection of plant material

The fresh leaves of *Echinops echinatus* were collected from Melghat region Dist-Amravati (Maharashtra) in the month July 2014 and the Authentication of plant was confirmed by Botanist (Prof. S.K Tippat, Department of Environment Science , Art, Commerce and Science College, Amravati).

Preparation of plant extract

The plant were dried over ambient temperature and the dried sample were grind properly and dried powder sample was extracted in Soxhlet extractor by using solvent Methanol at 65ºC. Extracts were concentrated by gradually evaporating the respective solvent on rotary evaporator[3]. The concentrated extract was collected in sterile bottles and kept in a cool and dark place prior to analysis[4].

Isolation of bioactive chemicals by column chromatography

Column chromatography was performed on a classic 20 cm long × 2 cm diameter glass column packed with silica gel Merck, Germany. The concentrated extract of *Echinops echinatus* (20 mL) was applied to the column by use of a pipette and the column was eluted sequentially with 90% Benzene and 10% Ethanol each fraction collected was tested prior GC-MS study.[2]

GC-MS Analysis of GC-MS Analysis of *Echinops echinatus*

Gas Chromatography and Mass Spectroscopy:

A JEOL GC mate II bench-top double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000[1] software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.[5]

Identification of chemical constituents:

Identification of the chemical constituents was done on the basis of retention index (RI) using a mass spectra library search NIST and by com-paring the mass spectral and retention data with literature [6]. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.[6,7]

RESULTS AND DISCUSION

The present study was carried out in methanolic extract of *Echinops echinatus* followed by column chromatography for isolation of bioactive constituents.. The GC–MS of isolated fractions by column chromatography of leaves extract of *Echinops echinatus* is shown in Table 1. The two major bioactive chemicals were found with % peak area 13-Docosenamide,(Z) (19.32), Phenol, 2,4bis (1,1dimethylethyl) (12.14) and Hexadecanoic acid, (8.32) The presence of biologically active molecules as major components in leaf extract makes it of high importance for medical purposes.

Table 1: Major chemical constituents in column fraction 1st

Sr. No	Retention Time	Name of chemical constituent	Molecular Formula	Molecular Weight	% Peak Area
1.	13.55	Phenol, 2,4bis(1,1dimethylethyl)	$C_{14}H_{22}O$	206.32	12.14
2.	23.26	Hexadecanoic acid,	$C_{19}H_{38}O_4$	256.42	8.32
3.	25.46	13Docosenamide,(Z)	$C_{22}H_{43}NO$	337.58	19.32

Gas Chromatogram Mass Spectrum of Column fraction-1 is shows by Figure 1 which indicates the presence of various chemical constituents i9n the crude extract of *Echinops echinatus* plant.

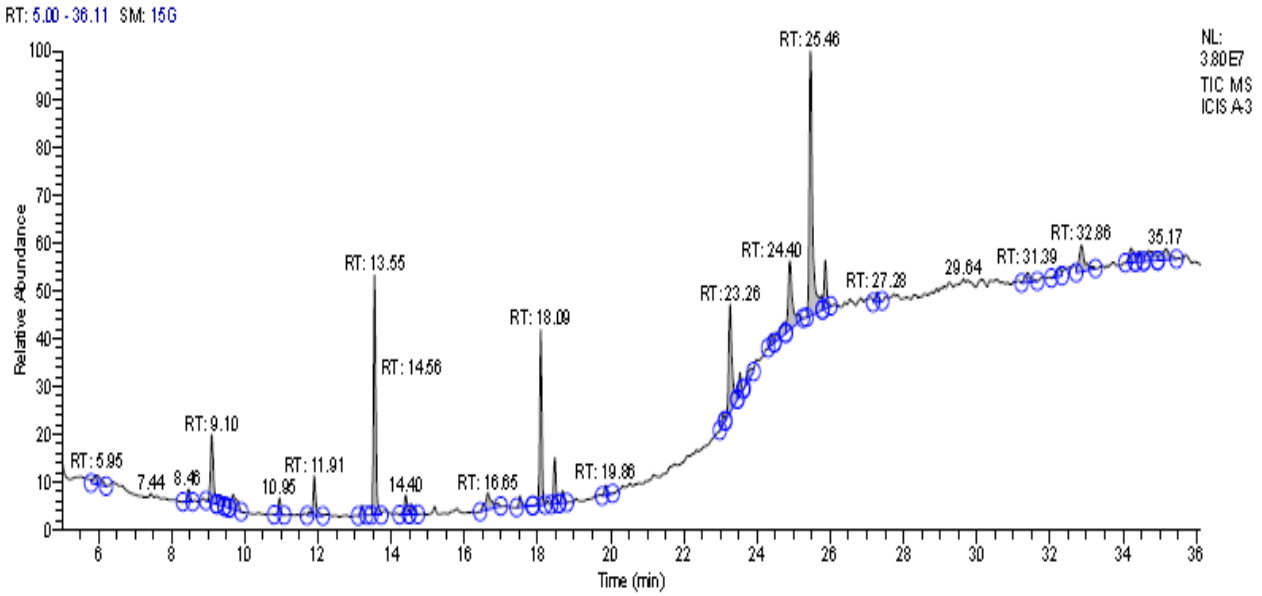
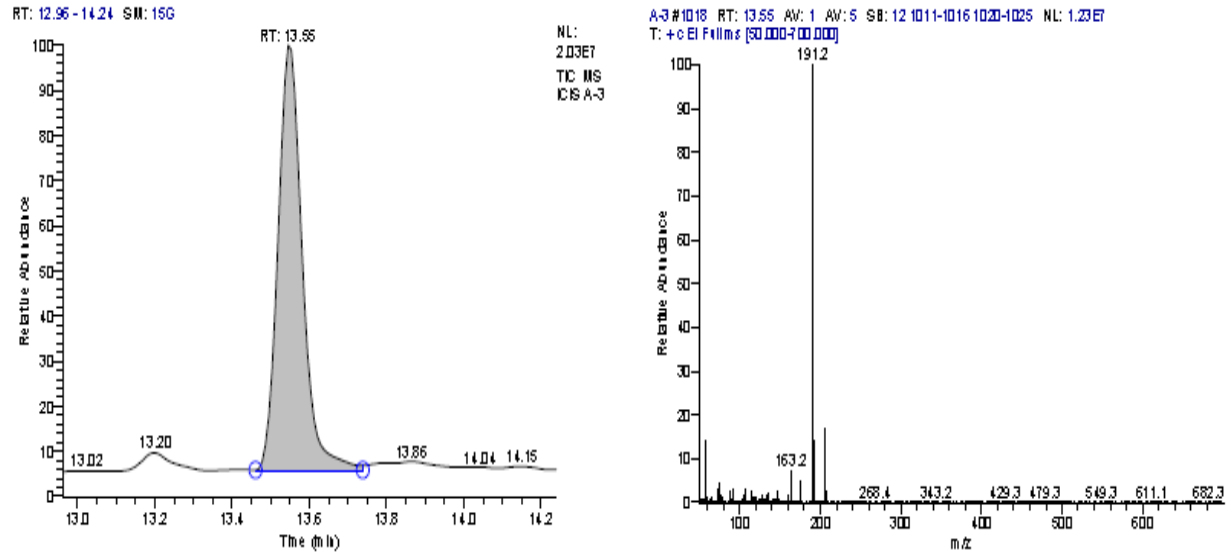


Figure 1: Gas Chromatogram Mass Spectrum of Column fraction-1

The extracted is further subjected for isolation of major components which is carried out by thin layer chromatography . Which is further analyzed by Gas Chromatography Mass Spectrum Figure 2 shows the presence of Phenol, 2,4bis(1,1dimethylethyl) $C_{14}H_{22}O$ at retention time of 13.55.

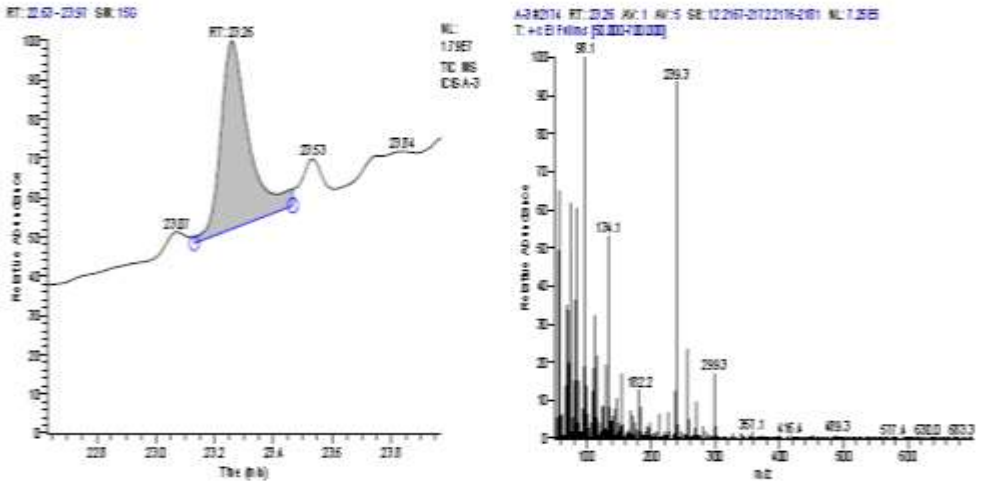


Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
Phenol, 2,4-bis(1,1-dimethylethyl)-	13.55	C14H22O	96-76-4

Figure 2: Mass Spectrum of Major peak at R.T- 13.55

Similarly Hexadecanoic acid is identified at retention time of 23.26 ($C_{19}H_{38}O_4$) which is clearly observed in Figure 3.

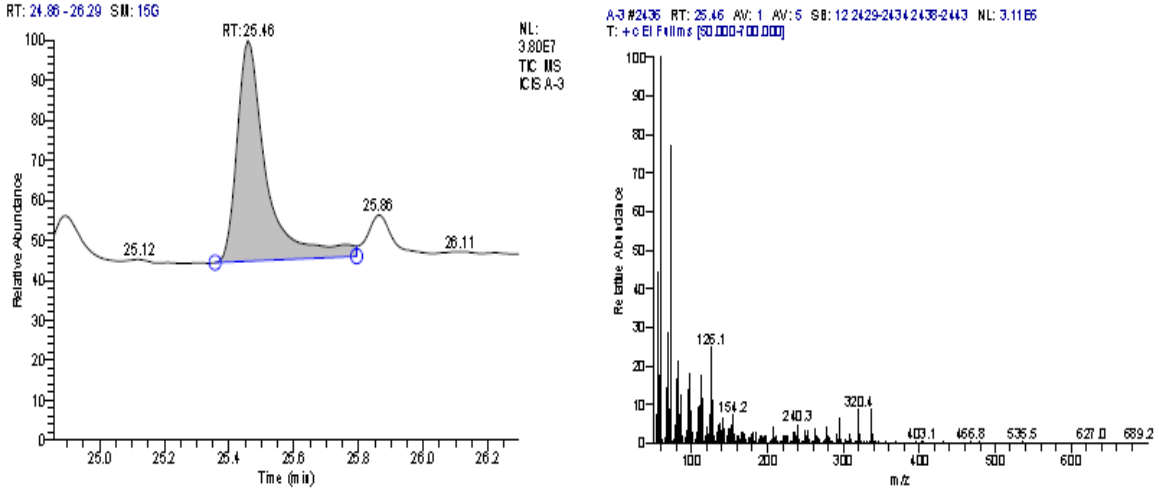


Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
Hexadecanoic acid,	23.26	C19H38O4	23470-00-0

Figure 3: Mass Spectrum of Major peak at R.T-23.26

Also, 13Docosenamide,(Z) ($C_{22}H_{43}NO$) is identified by Gas Chromatograph- Mass Spectrum at retention time of 25.46 which is shown in figure 4.



Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
13-Docosenamide, (Z)-	25.46	C22H43NO	112-84-5

Figure 4: Mass Spectrum of Major peak at R.T- 25.46

CONCLUSION

The results presented in this study are the first given information on the chemical composition of *Echinops echinatus.* It showed that 13 Docosenamide and Phenol, 2,4bis(1,1dimethylethyl) are the major fraction for the isolated method methods For future works, we will try to structural

elucidation by NMR analysis and carry out biological activity like antimicrobial, antioxidant and antifungal activity.

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