TLC and GC-MS analysis of Methanol extract of Root of Prosopis cineraria from Jaipur, Rajasthan, India

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Abstract: This study was aimed at analysis of potential bioactive constituents of root extracts of Prosopis cineraria Methanol were subjected to thin layer chromatography (TLC), Gas chromatography- mass spectroscopic (GC-MS) analysis. TLC analysis with Ethyl Methanol: Dichloromethane, solvent with Rf values 0.77, 0.75, 0.68, 0.58, 0.55, 0.35, 0.27 and 0.18. GC-MS analysis gave a spectrum of compounds with 15 major peaks corresponding to 3-Cyanobenzaldehyde, 1,4-BENZENEDICARBONITRILE, 2-Methoxy-4-vinylphenol, Ethyl 2-hydroxybenzyl sulfone, 17-OCTADECENOIC ACID, METHYL ESTER, 1,2-BENZENEDICARBOXYLIC ACID, Hexadecanoic acid, 9-Tricosene, Eicosane, Methyl 13-phenyl-tridecanoate, 1-Heptatriacotanol, 1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester, Squalene, Dotriacontane and Vitamin E. Most of which are bioactive compounds which may act as good antimicrobial, antiviral, antioxidant and anti-inflammatory agents.

Keywords: TLC, GC-Analysis, Prosopis cineraria, bioactive compounds.

Introduction

A plant extract contains large number of promising compounds, analysis of these compounds have become easier and more cost effective due to the development of Thin layer (TLC) and hyphenated chromatographic techniques such as GCMS, LC-MS (Sahaya et al., 2012). Thin layer chromatography is simple, quick and inexpensive process that can be used for analysis of mixtures (Fried and Sharma, 1994). GC-MS analysis is a breakthrough in analysis of phyto constituents and structure elucidation of these compounds as they have a sensitivity of detecting compounds as low as 1mg (Liebler et al., 1996). ***Prosopis cineraria*** is one of the general tree of the Indian desert belonging to family Mimosacease and locally famous as Khejri. This is a favorite tree for agro-forestry and is a trendy renewable source of fuel, fodder, timber and vegetables needed general population. It is a vital factor of desert environment of India as biomass manufacturer and as leguminous tree it enriches arid region soil, fix atmospheric nitrogen and provide a green exposure. The undeveloped and mature pods are energy rich safe to eat and have high nutritional value.

Materials and Methods

Plant collection and extraction

Plant sample of *Prosopis cineraria* was obtained from Jaipur, Rajasthan, India in the month of April 2015. This sample was authenticated and given identification number and submitted in Ethno-medicinal Herbarium, Centre with potentials of Excellence funded by DST, JECRC University, Jaipur, India. Further, voucher

specimens of *Prosopis cineraria* was deposited at herbarium of University of Rajasthan, Jaipur, India and was verified by senior taxonomist of department and provided with accession no. RUBL211578.

Figure No.01 *Prosopis cineraria* plant A. Leaf, B. Root and C. Stem

Extraction of the plant materials:

In the process of extraction of the plant material (root), selected species was dried and powdered to 100gm after that pretreated sample was placed in soxhelt apparatus for twelve to fourteen hours duration, the samples were extracted in petroleum ether, benzene, chloroform, ethyl acetate, methanol and distilled water. Then extracts were filtered, dried and weighed.

Thin layer Chromatographic analysis

TLC was performed on a pre-coated silica gel TLC plates grade F254 (E-Merck, Darmstadt, Germany) to determine the number of compounds present in the plant crude extract. A total of 5 l (10 mg/ml) of sample was spotted at 1 cm from the bottom of silica gel plates using capillary tubes. Development of the chromatogram was done in closed tanks, in which the atmosphere has been saturated with eluent vapour by wetting a filter paper lining. The chromatogram was visualized under UV light (365 nm and 254 nm), Sulfuric acid reagent spray. The R*f* values of the compounds were calculated using the following formula.

$$Rf = \frac{\text{distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

GC-MS Analysis

The extract was subjected to GC MS analysis to identify the various bioactive compounds present. The sample was analyzed in GCMS-QP2010 Plus from Delhi University (DU) India. . The volume of injected specimen was D of methanol extract, injector temp. 260°C with a split ratio of 10:0, Oven temperature program initial temp is 100°c. 250°C for 5 min, ramp 30 minute to 280°C, hold 69.98 min, ACQ Mode Scan: 40m/z to 600m/z, Column flow is 1.21mL/min and total flow is 16.3ml/min. Flow control with linear velocity which 40.9cm/sec. The identification of compounds was done using computer matching of mass spectra with those of standards (WILEY8. LIB. and NIST11.library). The name, molecular weight and the structure of the components of the test materials were ascertained. Figure No. 04

Results and Discussion

Thin layer chromatographic analysis revealed the presence of diverse potent biomolecules in the plant extract. Solvent system of MeoH :DCM (3:7) gave a better separation of

compounds (Fig.1 and Table1). Methanol extract revealed 8 spots with Rf values of 1. R_f 0.77 2. R_f 0.75 (Blue) 3. R_f 0.68 4. R_f 0.58 5. R_f 0.55, 6. R_f 0.35, 7. R_f 0.27 , 8. R_f 0.18. Figure no.02

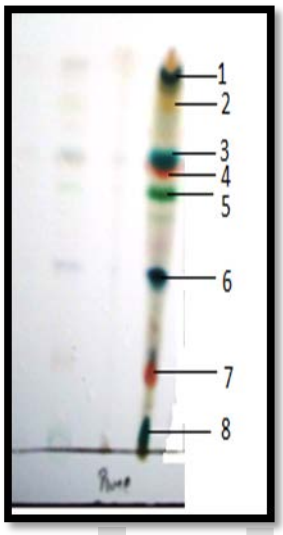


Figure 02. TLC profile of the active fraction compound present in MeoH extract of *Prosopis cineraria* (Root) [developing system: *n*-hexane : ethyl acetate]

The study on the active principles of Root of *Prosopis cineraria* by GCMS analysis showed the presence major peaks in Methanol extract corresponding to compounds 3-Cyanobenzaldehyde, 1,4-BENZENEDICARBONITRILE, 2-Methoxy-4-vinylphenol, Ethyl 2-hydroxybenzyl sulfone, 17-OCTADECENOIC ACID, METHYL ESTER, 1,2-BENZENEDICARBOXYLIC ACID, Hexadecanoic acid, 9-Tricosene, Eicosane, Methyl 13-phenyl-tridecanoate, 1-Heptatriacotanol, 1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester, Squalene, Dotriacontane and Vitamin E. The results of GCMS analysis was illustrated in **Table 01. Figure no. 03**

Table 01 List of compounds identified at various retention times from methanol extract of Root of *Prosopis cineraria* by GCMS

Peak	Retention time	Compound Name	Structure
1	8.444	3-Cyanobenzaldehyde	C8H5NO
2	8.648	1,4-BENZENEDICARBONITRILE	C8H4N2
3	9.822	2-Methoxy-4-vinylphenol	C9H10O2
4	11.215	Ethyl 2-hydroxybenzyl sulfone	C9H12O3S
5	21.284	17-OCTADECENOIC ACID, METHYL ESTER	C19H36O2
6	22.225	1,2-BENZENEDICARBOXYLIC ACID,	C26H42O4
7	22.475	Hexadecanoic acid,	C17H32O3
8	24.614	9-Tricosene,	C23H46

9	30.107	Eicosane	C20H42O
10	30.699	Methyl 13-phenyl-tridecanoate	C20H32O2
11	31.059	1-Heptatriacotanol	C37H76O
12	38.916	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C24H38O4
13	40.223	Squalene	C36H50
14	43.033	Dotriacontane	C32H66
15	46.160	Vitamin E	C29H50O2

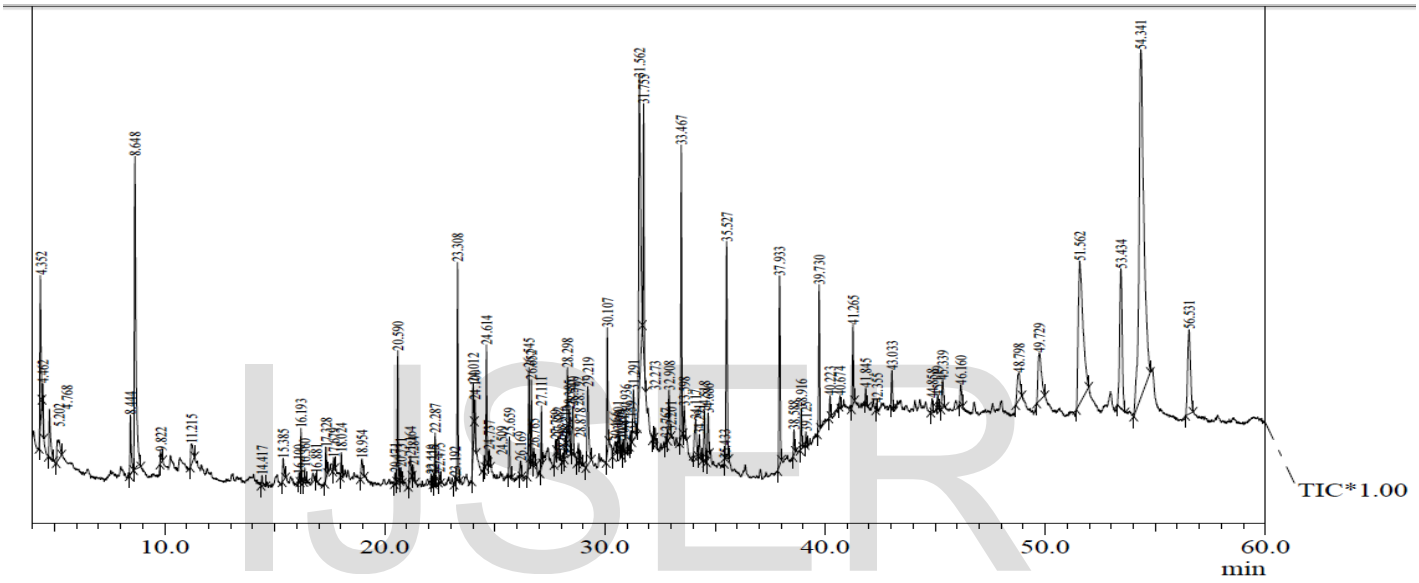
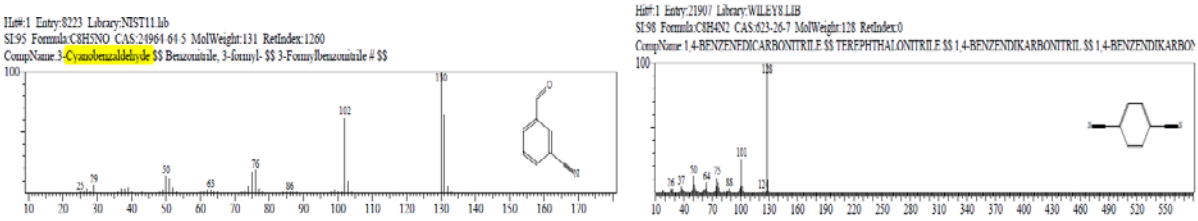
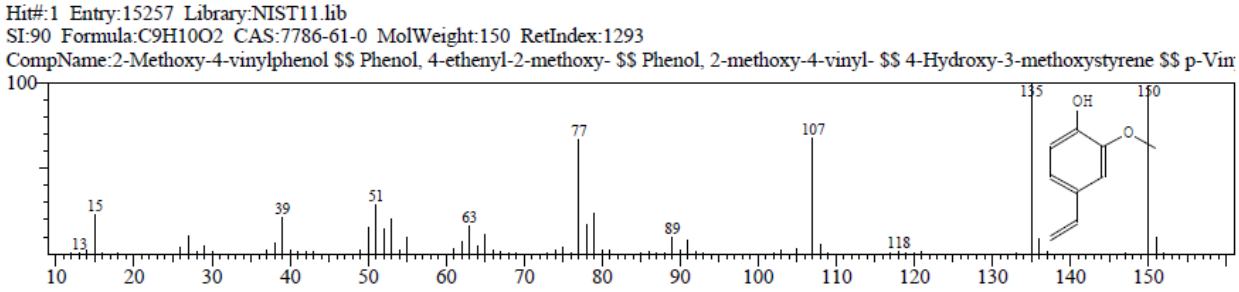
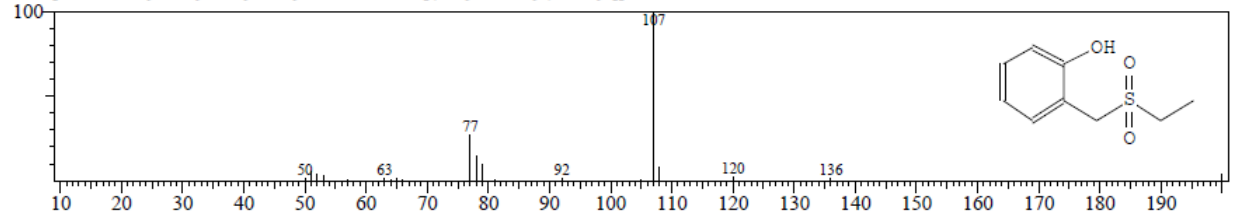


Figure.3 Mass Chromatogram of MeoH extract of Root of *Prosopis cineraria*.

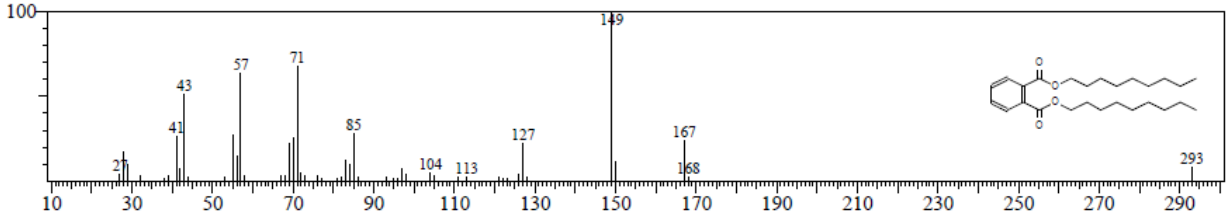




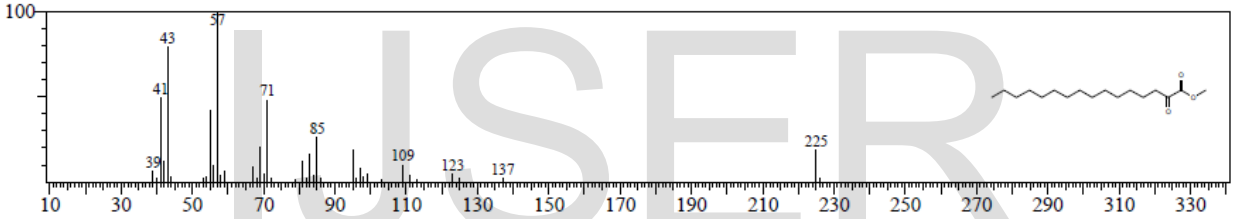
Hit#:1 Entry:43604 Library:NIST11.lib
SI:89 Formula:C9H12O3S CAS:53380-27-1 MolWeight:200 RetIndex:1720
CompName:Ethyl 2-hydroxybenzyl sulfone $$ 2-[(Ethylsulfonyl)methyl]phenol # $$



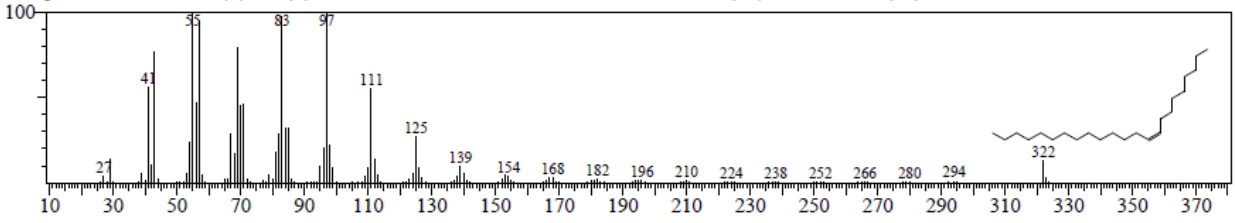
Hit#:7 Entry:346069 Library:WILEY8.LIB
SI:72 Formula:C26H42O4 CAS:84-76-4 MolWeight:418 RetIndex:0
CompName:1,2-BENZENEDICARBOXYLIC ACID, DINONYL ESTER $$ BISOFLEX 91 $$ BISOFLEX DNP $$ BISOLFLEX 91 $$ BRN 19



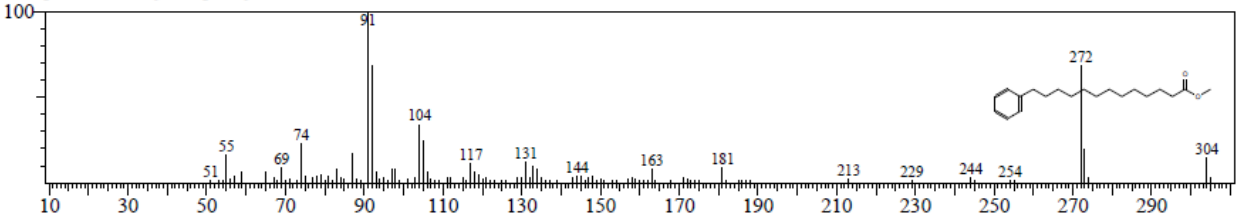
Hit#:1 Entry:106044 Library:NIST11.lib
SI:85 Formula:C17H32O3 CAS:55836-30-1 MolWeight:284 RetIndex:2014
CompName:Hexadecanoic acid, 2-oxo-, methyl ester $$ Methyl 2-oxohexadecanoate # $$



Hit#:1 Entry:135758 Library:NIST11.lib
SI:91 Formula:C23H46 CAS:27519-02-4 MolWeight:322 RetIndex:2315
CompName:9-Tricosene, (Z)- $$ (Z)-9-Tricosene $$ cis-9-Tricosene $$ Muscalure $$ (9Z)-Tricosene $$ (9Z)-9-Tricosene # $$



Hit#:1 Entry:121885 Library:NIST11.lib
SI:74 Formula:C20H32O2 CAS:0-00-0 MolWeight:304 RetIndex:2253
CompName:Methyl 13-phenyl-tridecanoate



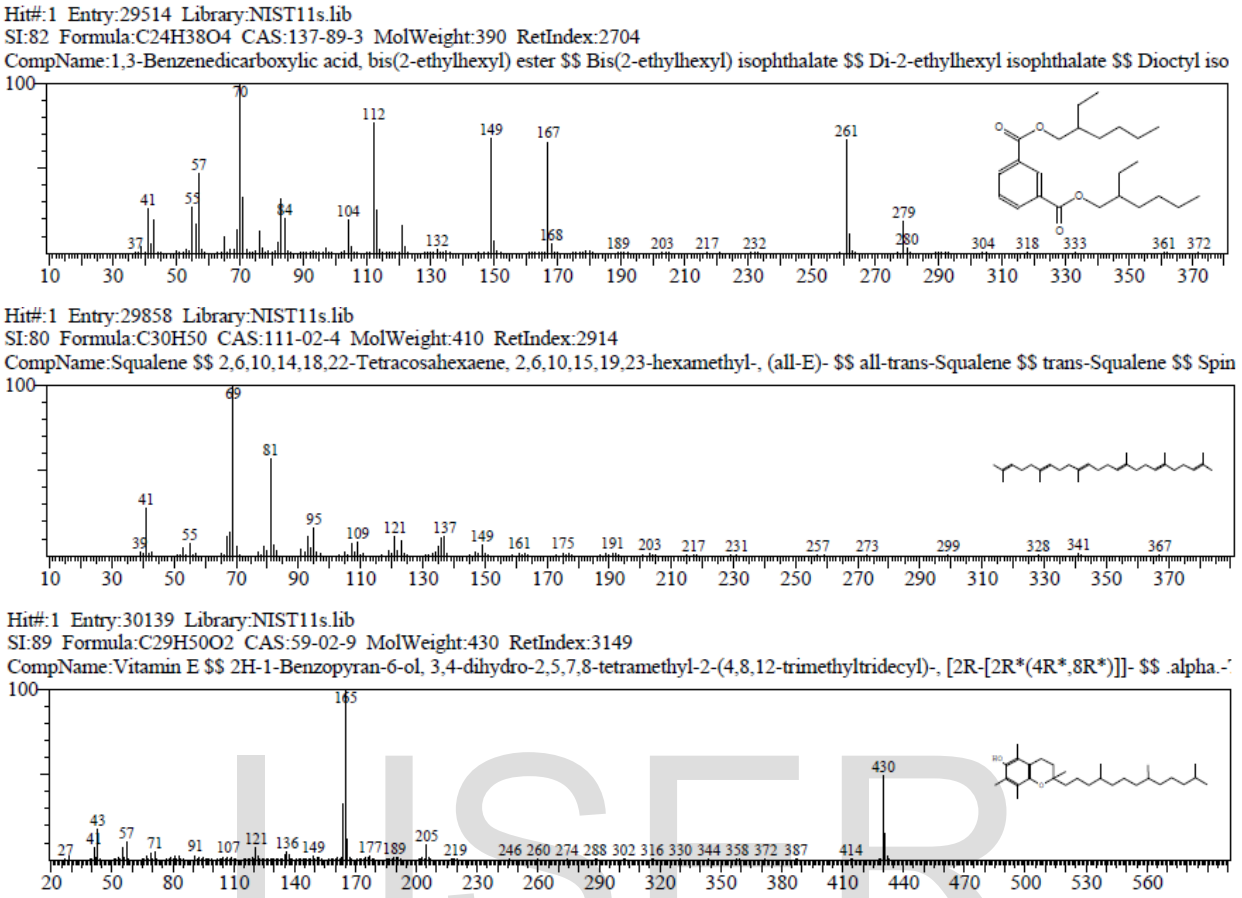


Figure No. 04 List of identified components from *Prosopis cineraria-Root extract with MeoH* are ascertained with WILEY8. LIB. and NIST11. Library

The results of the present study indicate that the methanol extract of Root of *Prosopis cineraria* have Isoflavonoids, carboxylic acids, fatty acids, Sesquiterpenes, steroids, phenolics which are potent phytochemicals with many biological activities. The presence of various bioactive compounds in the Root of *Prosopis cineraria necessarily* indicates its potential in treating various infectious diseases. Further research is necessary to identify and purify the compounds which can throw more light for better understanding and can open up new molecules with essential biological activity.

Conclusion

The result of the present investigation reveals that the methanol extracts of *Prosopis cineraria (Root)* possessed significant anticancer activity which was analyzed by GC-MS analysis. Squalene which was present in *Prosopis cineraria* may be responsible for anticancer activity. The GCMS analysis of the methanol extract of *Prosopis cineraria* reveals the presence of phytoconstituents belonging to the type acids, esters, alcohols, ethers, *etc.* Thus, the medicinal plant *Prosopis cineraria* is found to possess significant phytoconstituents.

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