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## PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF *PROSOPIS CINERARIA* PODS BY GC-MS

Garima Sharma, Devki, Ruchi Vyas and Rashmi Sisodia \*

Radiation Biology Laboratory, Department of Zoology, Centre for Advanced Studies, University of Rajasthan - 302004, Jaipur, India.

### **Keywords:**

P. cineraria,
Gas chromatography-mass
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### Correspondence to Author: Dr. Rashmi Sisodia

Professor,

Radiation Biology Laboratory, Department of Zoology, Centre for Advanced Studies, University of Rajasthan - 302004, Jaipur, India.

E-mail: rashsisodia@yahoo.co.in

**ABSTRACT:** This study was aimed to identify the possible bioactive chemical constituents of the state tree of Rajasthan, i.e., Prosopis cineraria L. (locally called Khejri) by GC-MS analysis using the GC-MS equipment (Shimadzu GCMSQP2010). For this purpose, the ethanolic extract of P. cineraria pods were used. Experimental conditions of the GC-MS system were as follows: Rtx-5MS capillary standard non-polar column, dimension: 30m, ID: 0.25 mm, Film: 0.25 µm was used, and flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. Crude samples that were dissolved in methanol were run fully at a range of 40-550 m/z, and the results were compared by using NIST Library. GC-MS analysis of an ethanolic extract of pods led to the identification of 32 compounds viz. Bicyclo[3.1.1]hept-3-ene, 4, 6, 6-trimethyl-2-vi (30.47%), 5-Isopropyl-2methylbicyclo [3.1.0] hexan-2-ol(19.38%), (-)-.beta. –Bourbonene (10.62%), 3-O-Methyl- d-glucose (5.04%), Kessane (4.01%), Thunbergol (3.65%), alfa.-Copaene (2.23%). There is not much information available on the phytochemical constituents of P. cineraria pods. This is the first attempt to investigate the GC-MS analysis of the ethanolic extract of this plant.

**INTRODUCTION:** The *P. cineraria* (L) Druce belonging to the family Leguminosae is an important tree (Khejri- a local name in Rajasthan) of the Thar Desert with hard climatic adaptation and is considered as one of the lifelines in desert habitat as mentioned in ancient literature. This is a species representing all five F *viz.*, Forest, Fiber, Fuel, Fodder, and Food. Pods of Khejri are eaten by cattle, sheep, horses, mules, donkeys, goats, camels, and other wildlife in the desert, especially blackbuck and chinkara in the western Rajasthan have survived by eating pods and leaves of this tree. This tree is also of mythological importance in local communities.



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The high value of this species has led it to be recognized as a State symbol (the state tree of Rajasthan). *P. cineraria* (L.) tree is endemic to the hot deserts of India. Pods of the tree are locally called "Sangri" and are considered as dry fruit of desert. It is one of the main ingredients of the quintessential Rajasthani dish "The Panchkuta".

Pods contain various phytoconstituents like tannins (gallic acid), steroids (campestral, stigmasterol, sitosterol, *etc.*), flavone derivatives (prosogerin A, B, C, D, and E), alkaloids (prosophylline, spicigerine) etc. which have been isolated from the sangria pod <sup>2</sup>. The fresh ripe pods contain 7-10% preformed water, and on a dry matter basis contain 9-17% crude protein, 1.2-4.3% ether extractives, 47-61% nitrogen-free extracts ,16-34% crude fibre, 28% acid detergent fibre, 8% acid detergent lignin, 4-5% ash, 0.14-0.29% silica, 0.3-0.5% calcium and 0.40-0.44% phosphorus. The qualitative chemical analysis of extracts of *Prosopis* pods was found positive for alkaloids, proteins, carbohydrates,

flavonoids, glycosides, saponins, and tannins in alcohol and aqueous solvent extracts. Also, 11 amino acids have been isolated from leaves and pods *viz*; aspartic acid, glutamic acid, serine, glycine, histidine, threonine, arginine, alanine, proline, valine, tyrosine, methionine, cysteine, isoleucine, leucine, phenylalanine, and lysine <sup>3</sup>.

*Prosopis* has been found to contain 5-hydroxy-tryptamine, apigenin, isorhamnetin-3-diglucoside, l- arabinose, quercetin, tannin, and tryptamine. The isolation of a flavone glycoside Patulitrin 3, 5, 6, 3, 4- pentamethoxy-7-hydroxy flavone from flowers of *P. cineraria* has been reported. The fruits of *P. juliflora* D.C. (leguminosae) were found to contain the same compound <sup>4</sup>. Many researchers found that patulitrin showed significant activity against the Lewis lung carcinoma *in-vivo* <sup>5</sup>.

P. cineraria pods contain protein, iron, vitamins A and C, and other micro minerals. Unripe pods are also nutritious and are used to prepare curries and pickles. P. cineraria (L) Druce is also a highly valued plant in the indigenous system of medicine. Its bark is said to be a potent drug for several ailments such as leprosy, dysentery, bronchitis, asthma <sup>6</sup>, leucoderma, piles, muscular tremors, asthma, rheumatism and inflammations. Pharmacological activities like analgesic, antipyretic, antihyperglycemic, anti-oxidant, antihypercholesterolemic, anti-tumor, nootropic, anthelmintic, anti-bacterial, anti-fungal, anti-viral, and anticancer activities have also been reported from different plant extracts <sup>3, 7-14</sup>. The inhibitory activities of enzymes lipid peroxidase, cyclooxygenase -1 and-2 in sangri pods was also reported <sup>15</sup>.

The chloroform fraction of stem bark of *P. cineraria* shows anti-diabetic activity in Steptozotocin induced diabetes in rats. The possible mechanisms of its anti-diabetic action include inhibition of carbohydrate hydrolytic enzymes, enhancement of glycogen regulatory enzymes expression in the liver and glucose uptake by tissues and adipocytes as well as stimulation of pancreatic insulin release <sup>16</sup>. Thus, *P. cineraria* have potential *in-vitro* and *in-vivo* anti-diabetic activity, and this effect could be due to multi-target mode of action that includes anti-hyperglycemic, post-prandial hypoglycemic, hypolipidemic, and insulin secretory actions <sup>17</sup>.

Phytoconstituents of an ethanolic pod extract of *P. cineraria* triggers the inhibition of HMG-CoA reductase, which is the main enzyme for cholesterol biosynthesis and the regression of atherosclerotic plaque in hyperchole-sterolemic rabbits <sup>18</sup>. *P. cineraria* pod extract components can be significantly considered in neuropharmacology as it showed inhibition against DPP-4, AChE, and BuChE target enzymes <sup>19</sup>.

After the GC-MS analysis of the methanolic extract of roots of *P. cineraria* showed the presence of significant phytoconstituents belonging to the type acids, esters, alcohols, ethers, *etc*. Significant anticancer activity was also reported. Squalene, which was present in *P. cineraria* may be responsible for anticancer activity <sup>20</sup>.

GC-MS analysis is a breakthrough in the analysis of phytoconstituents and structure elucidation of these compounds as they have a sensitivity of detecting compounds as low as 1 mg <sup>21</sup>. Therefore, we have done a GC-MS analysis for the identification of significant compounds in *P. cineraria* pods in the present study.

### MATERIALS AND METHODS:

Collection of Pods: The fruits of *P. cineraria* were collected from the Dausa district of Rajasthan, India, in the month of March 2017. The plant material was identified and authenticated by the Department of Botany, University of Rajasthan, Jaipur (RUBL 211667).

**Preparation of Plant Extract:** 25 gm of *P. cineraria* pods were air-dried in the shade at room temperature, and then dried pods were coarsely powdered. Plant material was extracted with 100ml ethanol by using a Soxhlet apparatus.

**GC-MS Analysis:** Five grams of sample was extracted successively with ethanol in Soxhlet extractor for 8 h. Brown colored residues were obtained after concentrating the extract under reduced pressure using a rotary evaporator.

The obtained extracts were stored in desiccators for further analysis. The dried sample was reconstituted in methanol to obtain 10 µgml<sup>-1</sup> concentrations. Methanol was used as a solvent. Finally, 2 ml of supernatant was taken and filtered through Axiva 0.2 µm nylon syringe filter and

transferred to GC vial for analysis. The analysis was carried out on a Shimadzu GCMSQP2010 Ultra system.

**GC-MS Conditions:** The injector temperature was 280 °C. The samples were injected in the split mode with a split ratio of 1/60. The injection volume was 1  $\mu$ L. A capillary column Rtx-5MS (5% Diphenyl-95% Dimethyl Polysiloxane), 30 m  $\times$  0.25 mm  $\times$  0.25 mm, was used. The carrier gas was helium

with a constant flow of 1.00 mL/min. The oven temperature was as follows: initial temperature of 60 °C, held for 2 min, increased to 10 °C/min up to 260 °C and held for 10 min. The MS ionization potential was 70 eV, and the temperatures were as follows: interface 260 °C, Ion source 280 °C. Mass scan range 40-550 m/z. GC-MS analysis of *P. cineraria* pods extracts led to the identification of 32 compounds **Table 1**.

TABLE 1: PHYTOCOMPONENTS IDENTIFIED IN THE ETHANOLIC EXTRACT OF P. CINERARIA PODS

| S. no. | Retention Time | Frequency Time |                | Area%  | Compound name  |
|--------|----------------|----------------|----------------|--------|--|
|        | 6.673          | 6.715          | Area<br>186598 | 0.31   | 1,3,5-Cycloheptatriene,3,7,7-trimethyl-                                      |
| 1      |                |                |                |        | -  |
| 2      | 7.227          | 7.275          | 242759         | 0.40   | 3-Carene   |
| 3      | 7.857          | 7.915          | 711648         | 1.18   | cis-4-methoxy thujane  |
| 4      | 8.224          | 8.295          | 1607340        | 2.67   | (1R,4R,5S)-1-Isopropyl-4-methoxy-4-  |
|        |                |                |                |        | methylbicyclo[3.1.0] hexane  |
| 5      | 8.571          | 8.655          | 326871         | 0.54   | Butanedioic acid, mono(3-phenyl-2-propenyl)                                  |
|        |                |                |                |        | ester  |
| 6      | 8.910          | 8.950          | 207457         | 0.35   | p-Mentha-1,5-dien-8-ol   |
| 7      | 9.016          | 9.055          | 351798         | 0.59   | Bicyclo[3.1.0]hexan-3-ol,4-methylene-1-(1-                                   |
| ,      | 9.010          | 9.033          | 331796         | 0.59   |  |
| 0      | 0.210          | 0.220          | 11650412       | 10.20  | methylethyl)-acetate   |
| 8      | 9.210          | 9.330          | 11650413       | 19.38  | 5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol                                 |
| 9      | 9.436          | 9.520          | 18312557       | 30.47  | Bicyclo[3.1.1]hept-3-ene, 4,6,6-trimethyl-2-                                 |
|        |                |                |                |        | vinyloxy   |
| 10     | 10.238         | 10.285         | 441547         | 0.73   | trans-Verbenol   |
| 11     | 10.449         | 10.510         | 399397         | 0.66   | (1,2,3-Trimethyl-cyclopent-2-enyl)-methanol                                  |
| 12     | 10.604         | 10.660         | 609895         | 1.01   | 2,4-Pentadienoicacid,3,4-dimethyl-,isopropyl                                 |
|        |                |                |                |        | ester  |
| 13     | 10.904         | 10.975         | 362245         | 0.60   | Carvacrol  |
| 14     | 11.582         | 11.625         | 361594         | 0.60   | (1R,3S,4R,5S)-1-Isopropyl-4-   |
|        |                |                |                |        | methylbicyclo[3.1.0] hexan-3-yl acetate -rel                                 |
| 15     | 11.982         | 12.025         | 457781         | 0.76   | alphaylangene  |
| 16     | 12.067         | 12.110         | 1340176        | 2.23   | alfaCopaene  |
| 17     | 12.193         | 12.330         | 6380761        | 10.62  | (-)betaBourbonene  |
| 18     | 12.450         | 12.515         | 361482         | 0.60   | betaLongipinene  |
| 19     | 12.785         | 12.860         | 829377         | 1.38   | cisalphaBergamotene  |
| 20     | 12.991         | 13.035         | 432706         | 0.72   | Germacrene D   |
| 21     | 13.355         | 13.390         | 1066554        | 1.77   | alfaCopaene  |
| 22     | 13.499         | 13.560         | 729162         | 1.21   | betaLongipinene  |
| 23     | 13.889         | 13.935         | 1103282        | 1.84   | Isoledene  |
| 24     | 14.100         | 14.180         | 2408796        | 4.01   | Kessane  |
| 25     | 14.643         | 14.740         | 1709613        | 2.84   | Diethyl Phthalate  |
| 26     | 15.623         | 15.745         | 3030384        | 5.04   | 3-O-Methyl-d-glucose   |
| 27     | 17.113         | 17.215         | 691809         | 1.15   | alphaPhellandrene, dimer   |
| 28     | 18.801         | 18.890         | 538651         | 0.90   | (R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-                                |
| 20     | 10.000         | 10.155         | 244766         | 0.55   | yl)cyclotetradeca-1,5,9-triene.  |
| 29     | 19.069         | 19.155         | 344766         | 0.57   | Isocembrol   |
| 30     | 19.566         | 19.625         | 305835         | 0.51   | 1,3,6,10-Cyclotetradecatetraene,3,7,11-trimethyl-                            |
| 21     | 20.556         | 20.620         | 400145         | 0.69   | 14-(1-methylethyl)   |
| 31     | 20.556         | 20.620         | 409145         | 0.68   | (R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl) cyclotetradeca-1,5,9-triene |
| 32     | 20.678         | 20.760         | 2190909        | 3.65   | Thunbergol   |
| 32     | 20.076         | 20.700         | 60103308       | 100.00 | 1 huhoei goi   |
|        |                |                | 00103308       | 100.00 |  |

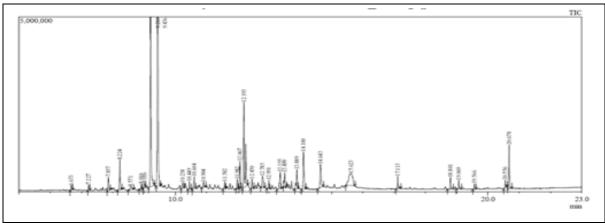
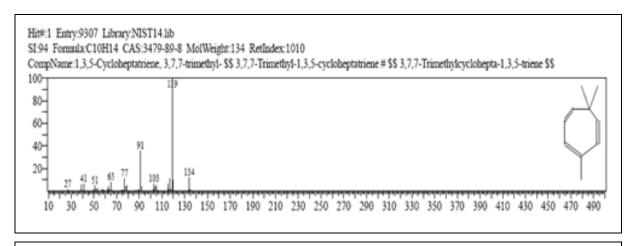
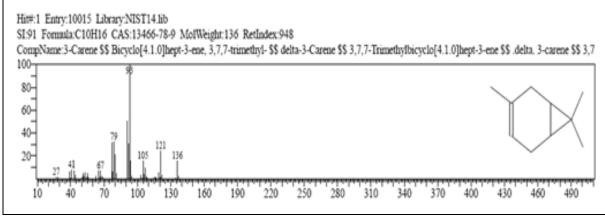
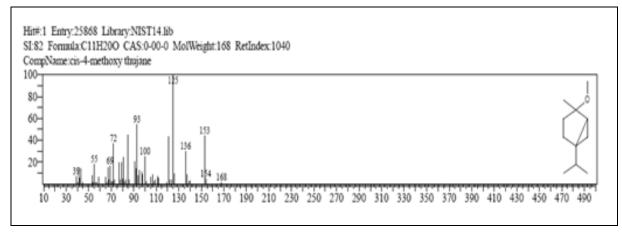
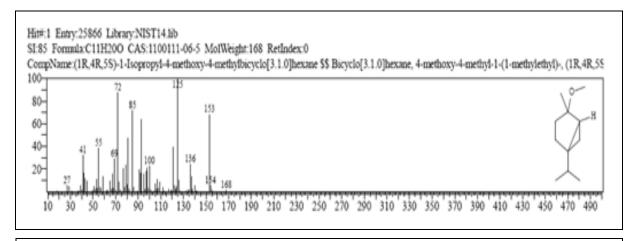


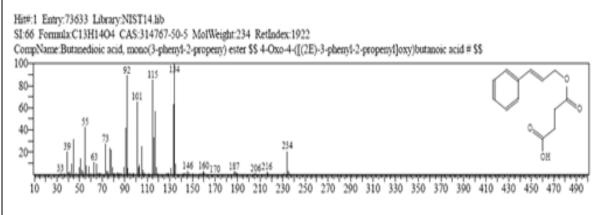
FIG. 1: GC-MS CHROMATOGRAM OF CONSTITUENTS OF ETHANOLIC EXTRACT OF PROSOPIS CINERARIA PODS

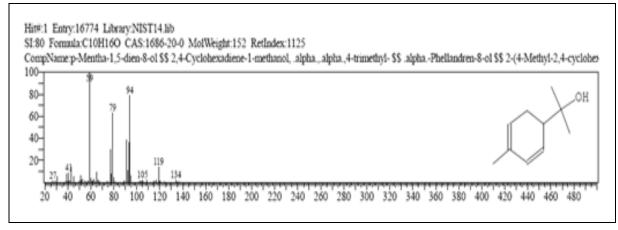


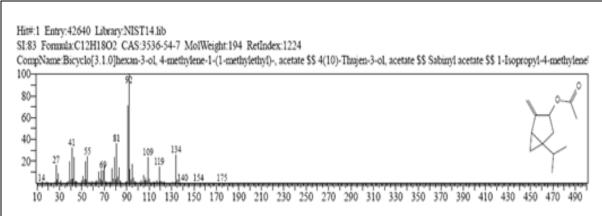


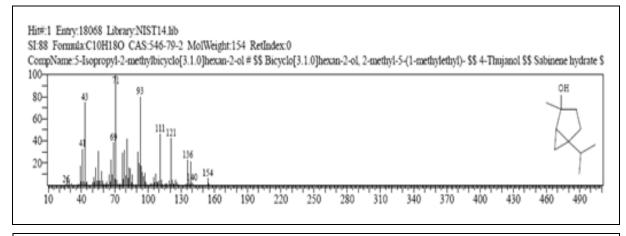


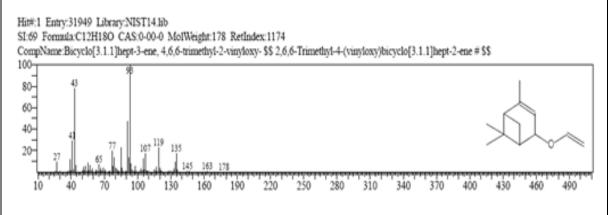


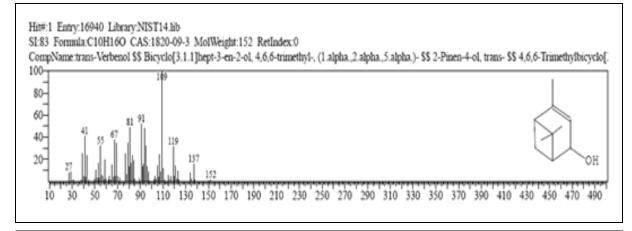


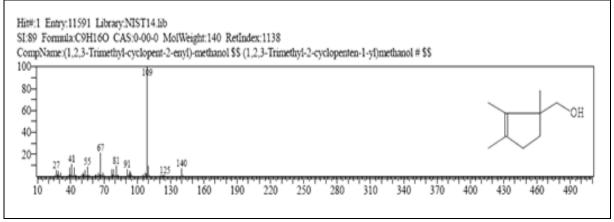


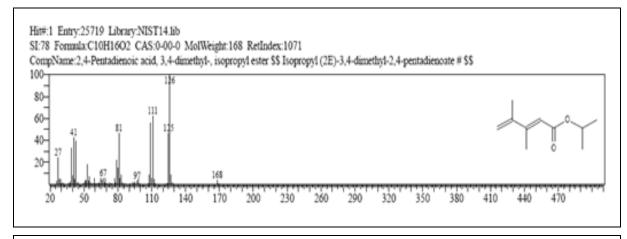


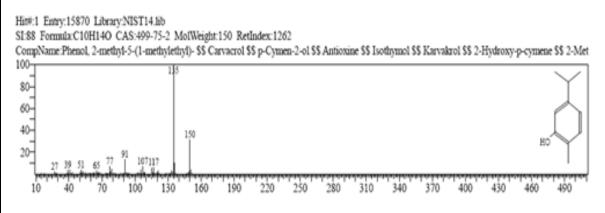


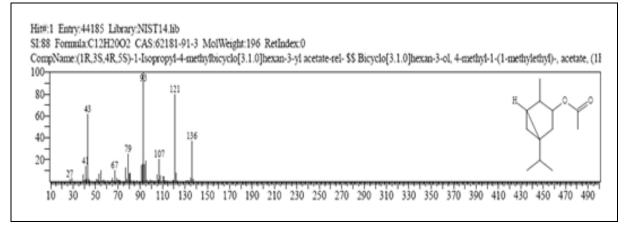


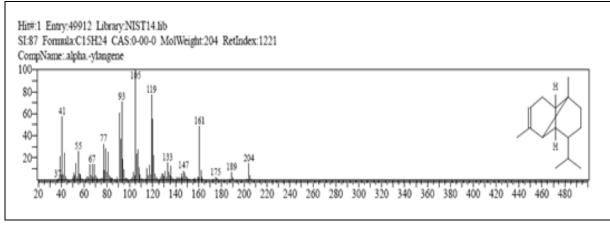


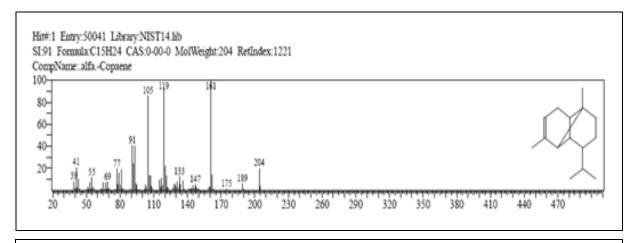


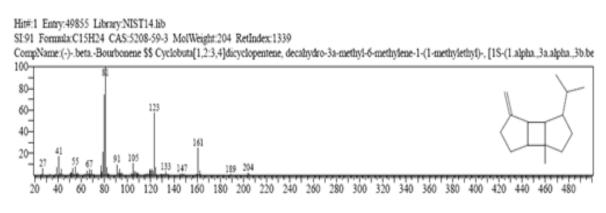


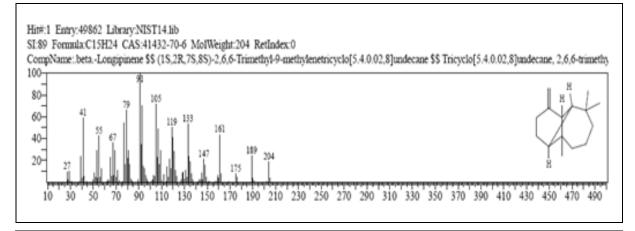


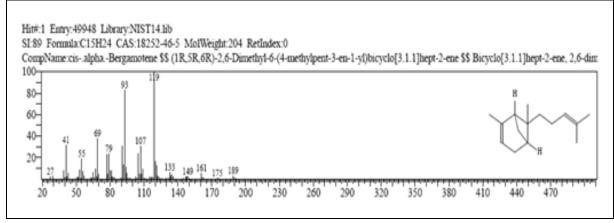


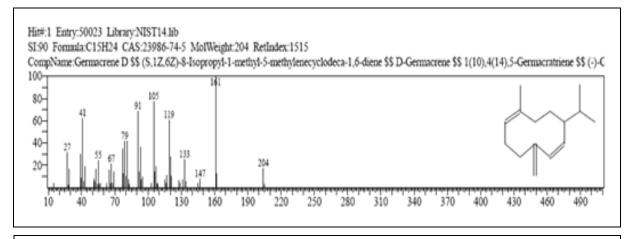


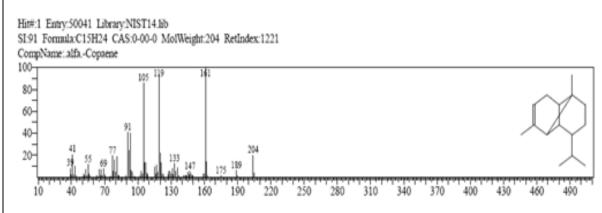


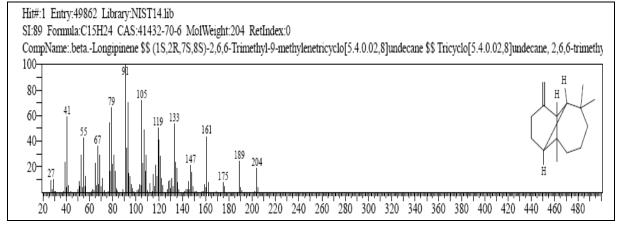


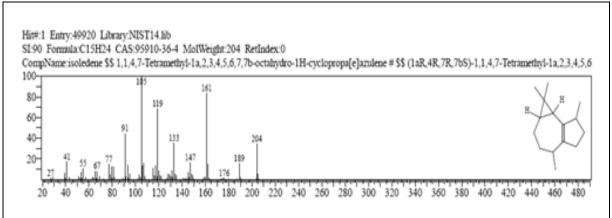


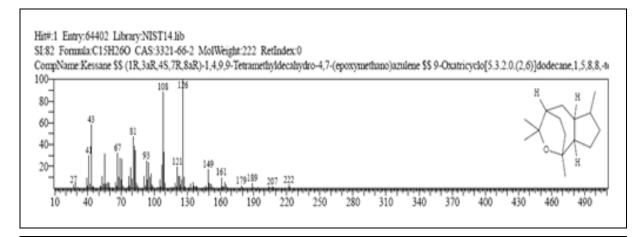


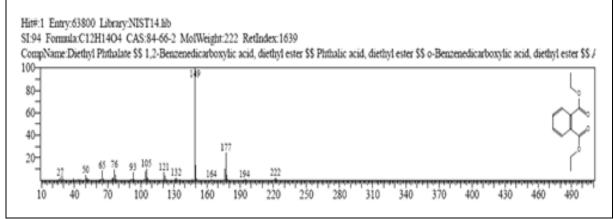


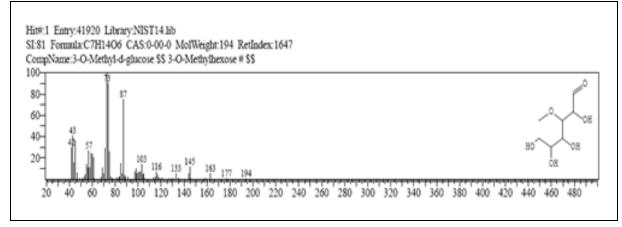


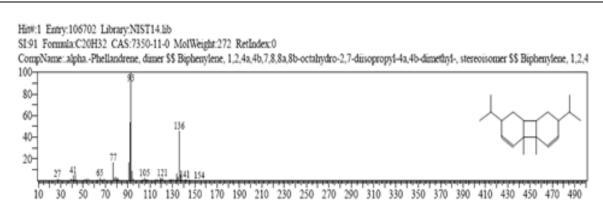


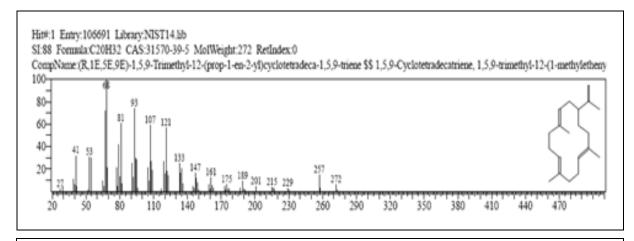


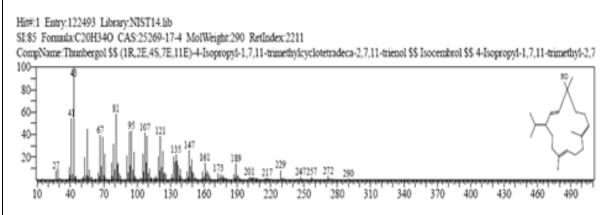


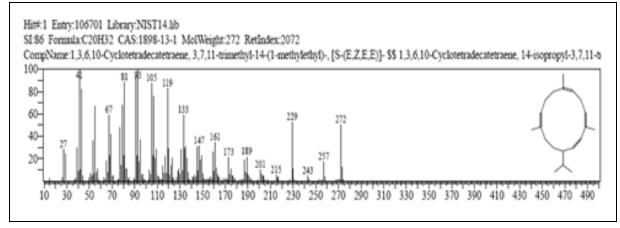


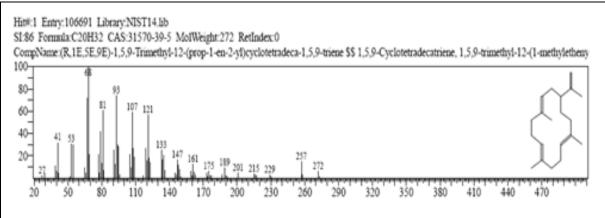












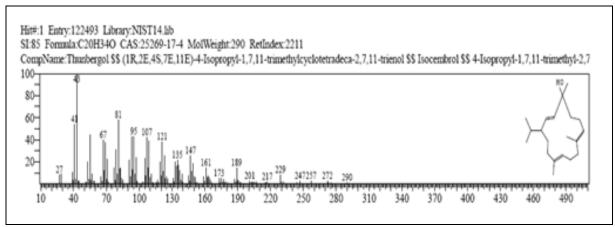


FIG. 2: LIST OF IDENTIFIED COMPOUNDS FROM P. CINERARIA PODS ARE ASCERTAINED WITH NIST14 LIBRARY

**RESULTS AND DISCUSSION:** The results pertaining to GC-MS analysis led to the identification of a number of compounds from the GC fractions of ethanolic extract of *P. cineraria*. These compounds were identified through mass spectrometry attached with GC. The results of the GC-MS was interpreted by using a database of the National Institute of Standards and Technology (NIST) library having more than 2,00,000 patterns. The GC-MS spectrum of the unknown constituent was then compared with the known components stored in NIST-14 library. The results of present study are tabulated in Table 1.

P. cineraria Pods Extract: GC-MS analysis of ethanolic extract of P.cineraria pods revealed the presence of 32 different phytocompounds namely1, 3, 5-Cycloheptatriene, 3, 7, 7-trimethyl (0.31%), 3-Carene (0.40%), cis-4-methoxy thujane (1.18%), (1R, 4R, 5S) -1-Isopropyl-4- methoxy-4-methylb (2.67%), Butanedioic acid, mono (3-phenyl-2propeny) (0.54%),p-Mentha-1,5-dien-8-ol (0.35%), Bicyclo [3.1.0] hexan-3-ol, 4-methylene-1- (1methylethyl) -acetate(0.59%), 5-Isopropyl-2-methylbicyclo [3.1.0] hexan-2-ol (19.38%), Bicyclo [3.1.1] hept-3-ene, 4, 6, 6-trimethyl-2-vi (30.47%), trans-Verbenol (0.73%), (1,2,3-Tri-methyl-cyclopent-2-enyl)- methanol (0.66%), 2, 4-Pentadienoic acid, 3, 4-dimethyl-, isopropy (1.01%), Carvacrol (0.60%), (1R, 3S, 4R, 5S)-1-Isopropyl-4- methylbicyclo [3.1.0] hexan- 3-yl acetate -rel (0.60%), alpha.-ylangene (0.76 %), .alfa.- Copaene (2.23%), (-)-. beta.- Bourbonene (10.62%), beta.- Longipinene (0.60%), cis-.alpha.-Bergamotene (1.38%), Germacrene D (0.72%), alfa.- Copaene (1.77%), beta.-Longipinene (1.21%), Isoledene (1.84 %), Kessane (4.01%), Diethyl Phthalate (2.84%), 3-O-Methyl-dglucose (5.04%),. alpha.- Phellandrene, dimer (1.15%), (R, 1E, 5E, 9E)-1, 5, 9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1, 5, 9-triene (0.90%), Isocembrol (0.57%), 1, 3, 6, 10-Cyclotetra-decatetraene, 3, 7, 11-trimet (0.51%), (R, 1E, 5E, 9E)-1, 5, 9-Trimethyl-12- (prop-1-en-2-yl) cyclo-tetradeca-1, 5, 9-triene (0.68%), Thunbergol (3.65%).

TABLE 2: PHARMACEUTICAL / THERAPEUTIC POTENTIAL OF THE PHYTOCOMPONENTS IDENTIFIED IN THE ETHANOLIC EXTRACT OF *P. CINERARIA* PODS

| Phytoconstituents      | Properties                                     |  |  |
|------------------------|--|--|--|
| identified in P.       |  |  |  |
| cineraria pods         |  |  |  |
| 3-Carene               | Anti-inflammatory                              |  |  |
|                        | properties <sup>22,23</sup>                    |  |  |
|                        | Anti-oxidant, anti-stress <sup>24</sup>        |  |  |
|                        | and antifungal activity 25                     |  |  |
| Diethyl phthalate,     | antimicrobial activity 26                      |  |  |
| Carvacrol, Thymol      |  |  |  |
| trans-Verbenol         | Anti-bacterial activity <sup>27</sup>          |  |  |
| alphaylangene          | Anti-oxidant activity <sup>28</sup>            |  |  |
| alfa-Copaene           | analgesic and                                  |  |  |
|                        | anti-inflammatory <sup>29</sup>                |  |  |
| betaBourbonene         | Anti-tumour,                                   |  |  |
|                        | Anti-inflammation and                          |  |  |
|                        | anti-fungal effects 30                         |  |  |
| betaLongipinene        | anticancer properties 31                       |  |  |
| cis- alpha-Bergamotene | herbivore-induced plant volatile <sup>32</sup> |  |  |
| Germacrene-D,          | cytotoxic nature 33,34,35                      |  |  |
| Isoledene              |  |  |  |
| Kessane                | Anti-oxidant properties <sup>36</sup>          |  |  |
| alphaPhellandrene      | Hydroxyl radical                               |  |  |
|                        | scavenger 37                                   |  |  |
| Isocembrol             | Anti-oxidant activity 38                       |  |  |
|                        | neuro-protective and anti-                     |  |  |
|                        | cancer activity 39                             |  |  |
|                        | Anti-microbial and                             |  |  |
| Thunbergol             | Larvicidal properties <sup>40</sup>            |  |  |

The GC-MS spectrum confirmed that the presence of 32 major components with the retention time

6.673, 7.227, 7.857, 8.224, 8.571, 8.910, 9.016, 9.210, 9.436, 10.238,10.449, 10.604, 10.904, 11,582, 11.982, 12.067, 12.193, 12.450, 12.785, 12.991, 13.355, 13.499, 13.889, 14.100, 14.643, 15.623, 17.113, 18.801, 19.069, 19.566 respectively **Fig. 1**. The name, molecular weight, molecular formula and structure of the component of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The identified phytoconstituents from pods of P. cineraria possess the following properties **Table 2**.

**CONCLUSION:** In the present study, the GC-MS analysis of an ethanolic extract of pods of *P. cineraria* revealed the presence of thirty-two compounds. Such pods are consumed as the dietary agent and may be further explored for their medicinal potential as the phytoconstituents identified has potent anticancer and antioxidant activities which can be used to reduce the damages caused by free radicals in the body and therefore contribute to the prevention of diseases related to the oxidative stress and also for the management of diabetes, inflammation and microbial infections.

Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principle bioconstituents in this medicinal plant. The identification of phytoconstituents would be helpful for further detailed studies.

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