

## IDENTIFICATION OF SOME BIOACTIVE CONSTITUENTS OF GLYCOSMIS PENTAPHYLLA RETZ. DC. USING GC-MS

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### ABSTRACT

*Glycosmis pentaphylla* commonly known as “Anam” in Tamil is a medicinally important plant belonging to the family Rutaceae. The leaves are used in traditional medicine for the treatment of fever, hepatopathy, helminthiasis, wounds, eczema, erysipelas and other skin diseases. The fruits are useful in vitiated conditions of vata, pitta and kapha. The roots are effective in treating facial inflammations, rheumatism, jaundice and anaemia. A study was conducted with the objective of identifying some new chemical constituents present in the ethanolic extract of the roots of *Glycosmis pentaphylla* by subjecting it to GC-MS analysis. Twenty five chemical constituents have been identified, of which six were for the first time identified major

constituents namely 7H-Furo (3,2-G) (1)Benzopyran-7-one,2,3 – dihydro – 2 - (1-Hydroxy-1methylethyl) - (s) - (10.96%), Stimast-5-en3-yl-9-octadecenoate (9.06%), Gama.-Sitosterol (8.84%), 5-hydroxypipelic acid(6.23%), (-)-Guaiol (5.58%), 1H-Indole-3-Ethanamine, 5-Methoxy-N, 1- Dimethyl (5.81%).

**Keywords:** *Glycosmis pentaphylla*, ethanol root extract, phytochemicals, GC-MS analysis.

### INTRODUCTION

*Glycosmis pentaphylla* Retz.DC (Rutaceae) commonly known as Anam, Kula pannai in Tamil is found all over India, China, Malaysia, Sri lanka, Borneo and Australia and has

historically been used to treat wounds, inflammation, rheumatism, jaundice and anaemia.<sup>1,2</sup> The plant is known by various names in different languages as Vananimbuka in Sanskrit, Ban-nimbu in hindi, Manikyan in kannada, Panal in Malayalam and Gongi pandu in Telugu. Traditionally *Glycosmis pentaphylla* is reported to possess number of medicinal properties. The whole plant is used as an astringent, vermifuge, febrifuge, anti inflammatory, expectorant, fever, helminthiasis, cough, bronchitis, rheumatism, jaundice, hepatopathy, eczema, skin diseases, wounds and erysipelas.<sup>3</sup> The plant is also being used for the treatment of anaemia in the folklore medicine. Around 40 biologically active constituents from various parts of this plant were identified using different solvent systems and reported in literature. Studies carried out on the leaves and twigs of *Glycosmis pentaphylla* have reported two furoquinoline bases kokusaginine and skimmianine<sup>4</sup>. A carbazole derivative named glycozoline was isolated from bark<sup>5</sup> and a quinazoline alkaloid base glycophynoline was isolated from flowers of the plant<sup>6</sup>. Studies done on the seeds of the plant have reported isolation of a carbazole derivative of glycozoline.<sup>7</sup> Though studies have been carried out on the root part of the plant and carbazole alkaloids of glycozolidal have been reported<sup>8</sup>, not much have been explored on the constituents of the root of the plant. Hence a systematic and scientific study was done to identify some new phytochemical constituents from the ethanolic extract of the roots of *Glycosmis pentaphylla* using GC-MS technique.

## MATERIALS AND METHODS

### Collection of Plant Material

The roots of *Glycosmis pentaphylla* was collected in the month of January from Tirumala hills, Tirupathi, Andhra Pradesh, Western ghats of South India. The plant was identified by Plant Anatomy Research Center, West Tambaram, Chennai, Tamil Nadu, India. A voucher specimen has been kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai-600 116, Tamil Nadu, India.

### Preparation of Powder and Extract

The root was shade dried and pulverized in a mechanical grinder. The powder (1.5 kg) was successively extracted with various solvents such as petroleum ether (40-60<sup>0</sup>C), chloroform, ethyl acetate, ethanol, 50 % hydro alcohol. The extracts were concentrated under reduced pressure in a rotary evaporator (Technico, India). The successive ethanolic extract of the root of *Glycosmis pentaphylla* was used for GC-MS analysis.<sup>9</sup>

## GC-MS analysis

### Preparation of Sample

2 µl of the ethanolic root extracts of *Glycosmis pentaphylla* was employed for GC-MS analysis.

### Instruments and chromatographic conditions

GC-MS analysis was carried out on a GC-MS-QP 2010 [SHIMADZU] system comprising a autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column VF-5ms fused silica capillary column (30 × 0.25 x0.25) operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and Interface temp: 240°C, Scan range : 40 – 1000 m/z

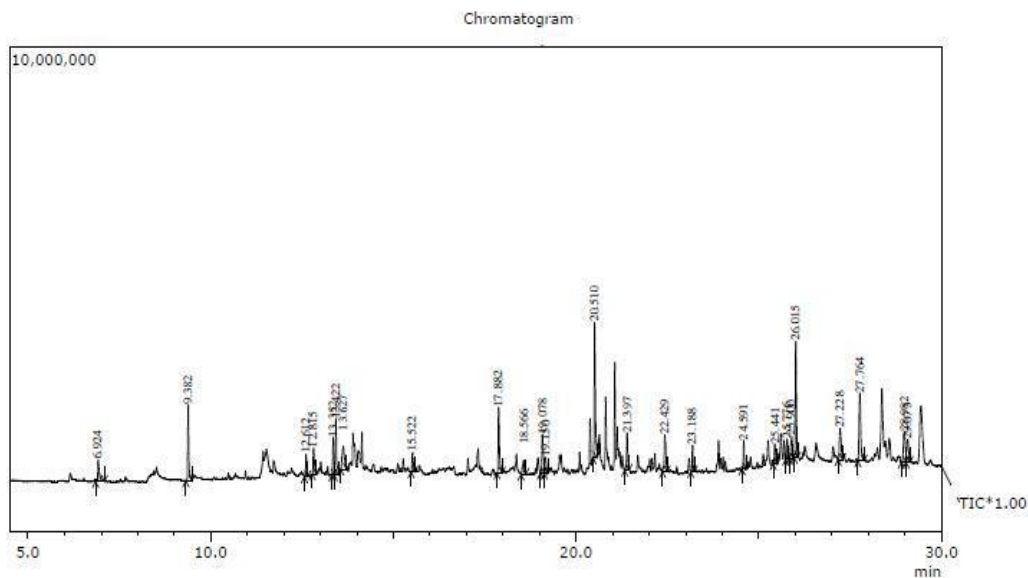
Solvent cut time: 5mins, MS start time : 5(min), MS end time : 35 (min), Scan speed:2000  
Mass spectra were taken at 70 eV; Scan speed : 2000

## RESULTS AND DISCUSSION

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST), WILEY 8 and FAME having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.<sup>10,11</sup> The identified compounds were tabulated in the table no.1 and the literature study reveals that six of the phytochemical constituents were found to be major and its mass spectra were presented in Figure -1 to Figure – 7; they were identified as 7H-Furo (3,2-G)(1)Benzopyran-7-one,2,3-dihydro-2-(1-Hydroxy-1methylethyl)-,(s)-(10.96 %), Stimast-5-en3-yl-9-octadecenoate(9.06%), Gama.-Sitosterol (8.84%), 5-hydroxypipelic acid(6.23%), (-)-Guaiol (5.58%), 1H-Indole-3-Ethanamine,5-Methoxy-N,1-Dimethyl(5.81%) respectively. The compound 7H-Furo (3,2-G)(1)Benzopyran-7-one,2,3-dihydro-2-(1-Hydroxy-1methylethyl)-,(s)- also known as marmesin is a dihydro furocoumarin derivative. The present study had identified and reported for the first time marmesin from the ethanolic extract of the *Glycosmis pentaphylla*. The study is to be further extended to isolate marmesin from the ethanolic extract of the root of *Glycosmis pentaphylla*.

**Table.1. Identified phytocomponents in the ethanolic extract of the root of *Glycosmis pentaphylla* by GC-MS**

Peak Number	Retention Time	Peak Area Percentage	Name of the compound	Molecular Formula	Molecular Weight
1	6.924	2.55	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144
2	9.328	6.23	5-hydroxypipicolic acid	C <sub>6</sub> H <sub>11</sub> NO <sub>3</sub>	145
3	12.612	1.55	Cyclohexanemethanol,4-ethyl- $\alpha$ ,4-trimethyl-3-(1-methylethyl)-,(1R-(1. $\alpha$ .,3 $\alpha$ .,4 $\beta$ .)	C <sub>15</sub> H <sub>26</sub> O	222
4	12.815	2.51	3-tert-butyl-4-hydroxyanisole	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	180
5	13.352	2.83	Rosifoliol	C <sub>15</sub> H <sub>24</sub> O	220
6	13.422	5.58	(-)-Guaiol	C <sub>15</sub> H <sub>26</sub> O	222
7	13.627	4.22	Beta-D-Glycopyranoside,methyl	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194
8	15.522	1.95	N,N-Dimethyltryptamine	C <sub>12</sub> H <sub>16</sub> N <sub>2</sub>	188
9	17.882	5.81	1H-Indole-3-Ethanamine,5-Methoxy-N,1-Dimethyl	C <sub>13</sub> H <sub>18</sub> N <sub>2</sub> O	218
10	18.566	1.86	Octadecanoic acid, Methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298
11	19.078	4.36	Hexadecanoic acid ,butyl ester	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312
12	19.150	1.88	1-tricosene	C <sub>23</sub> H <sub>46</sub>	322
13	20.510	10.96	7H-Furo(3,2-G)(1)Benzopyran-7-one,2,3-dihydro-2-(1-Hydroxy-1methylethyl)-,(s)-	C <sub>14</sub> H <sub>14</sub> O <sub>4</sub>	246
14	21.397	3.13	1,2-Propanediol,3-benzyloxy-1,2-diacetyl-	C <sub>14</sub> H <sub>18</sub> O <sub>5</sub>	266
15	22.429	4.46	Dehydroabietic acid	C <sub>23</sub> H <sub>36</sub> O <sub>2</sub> Si	372
16	23.188	2.07	Nonacosane	C <sub>29</sub> H <sub>60</sub>	408
17	24.591	2.28	Hexatriacontane	C <sub>36</sub> H <sub>74</sub>	506
18	25.441	1.84	Ergost-5-en-3-ol,(3. $\beta$ .)-	C <sub>28</sub> H <sub>48</sub> O	400
19	25.776	1.46	Stigmast-5-en-3-ol,oleate	C <sub>29</sub> H <sub>50</sub> O	414
20	25.900	2.29	n-Tetraetracontane	C <sub>44</sub> H <sub>90</sub>	618
21	26.015	9.09	Stimast-5-en-3-yl-9-octadecenoate	C <sub>47</sub> H <sub>82</sub> O <sub>2</sub>	678
22	27.228	4.17	Stigmastrol	C <sub>29</sub> H <sub>48</sub> O	412
23	27.764	8.84	Gama.-Sitosterol	C <sub>29</sub> H <sub>50</sub> O	414
24	28.982	4.77	Butyl 11-elcosenoate	C <sub>24</sub> H <sub>46</sub> O <sub>2</sub>	366
25	29.073	3.33	(24R)-4-Stigmasten-3-one	C <sub>29</sub> H <sub>48</sub> O	412



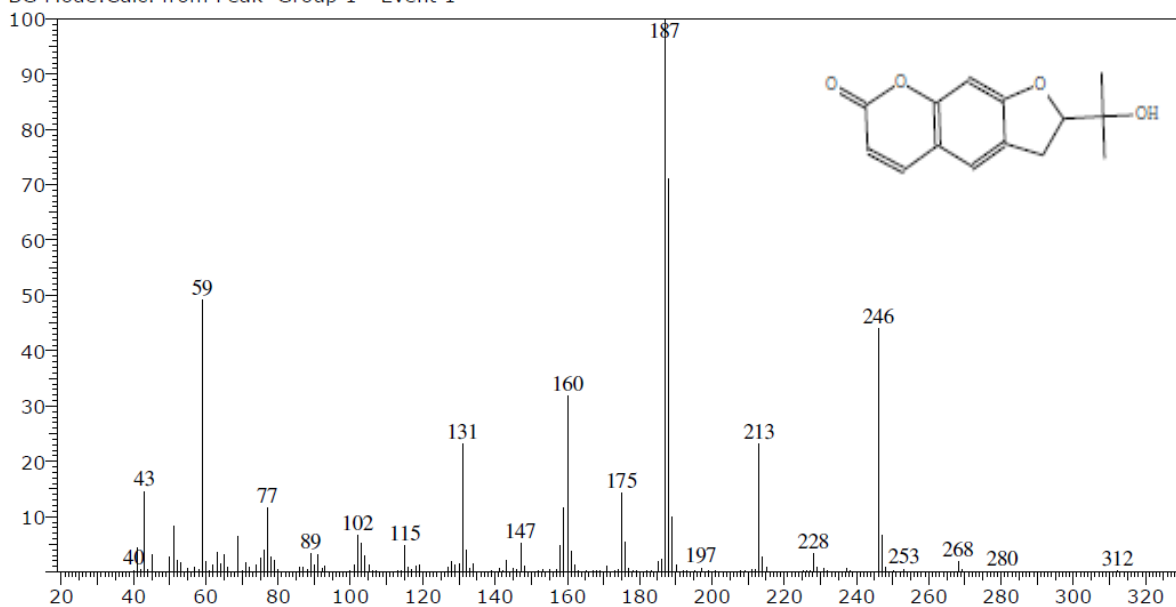
**Figure.1.GC-MS Chromatogram of ethanolic extract of the root of *Glycosmis entaphylla*.**

Line#:13 R.Time:20.508(Scan#:2102)

MassPeaks:173

RawMode:Averaged 20.500-20.517(2101-2103) BasePeak:187(436929)

BG Mode:Calc. from Peak Group 1 - Event 1



**Figure.2.H-Furo(3,2-G)(1)Benzopyran-7-one,2,3-dihydro-2-(1-Hydroxy-1methylethyl)-, (s)- (RT: 20.510)**

Line#:21 R.Time:26.017(Scan#:2763)

MassPeaks:277

RawMode:Averaged 26.008-26.025(2762-2764) BasePeak:396(128412)

BG Mode:Calc. from Peak Group 1 - Event 1

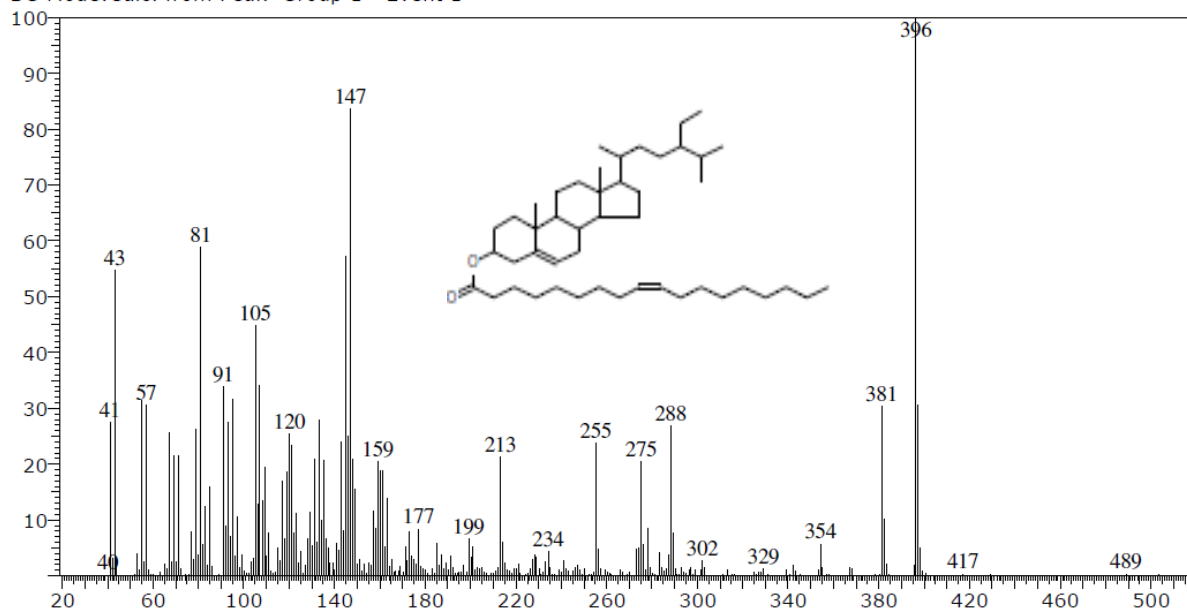


Figure.3. Stimast-5-en3-yl-9-octadecenoate (RT: 26.015)

Line#:23 R.Time:27.767(Scan#:2973)

MassPeaks:283

RawMode:Averaged 27.758-27.775(2972-2974) BasePeak:414(50767)

BG Mode:Calc. from Peak Group 1 - Event 1

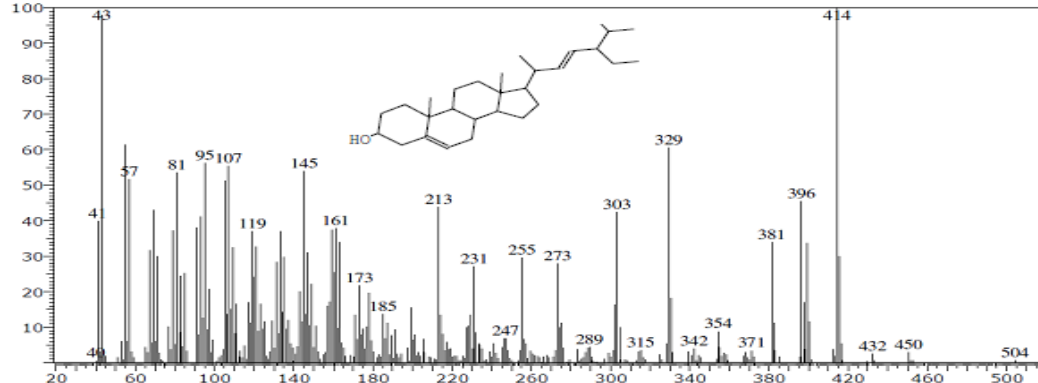


Figure.4.Gama.-Sitosterol (RT: 27.764)

MassPeaks:71

RawMode:Averaged 9.375-9.392(766-768) BasePeak:100(687421)

BG Mode:Calc. from Peak Group 1 - Event 1

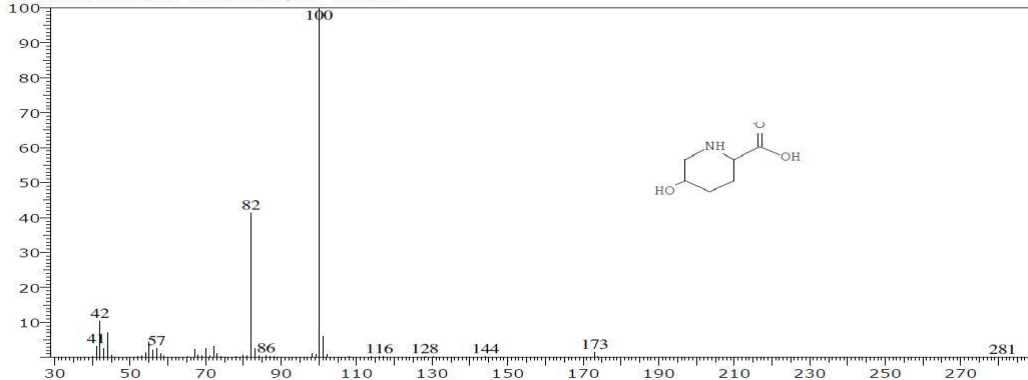
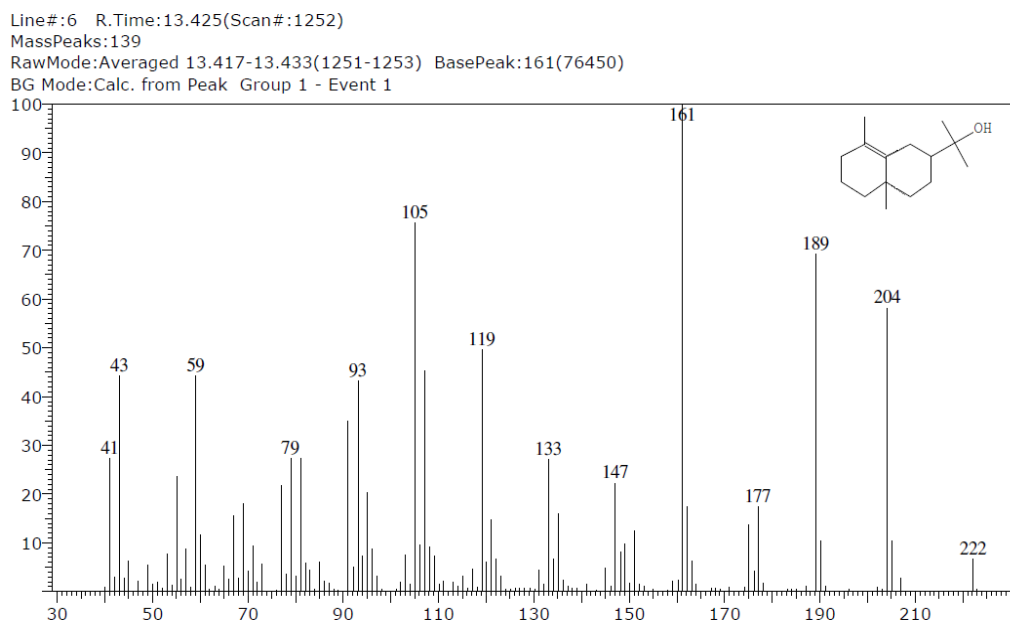
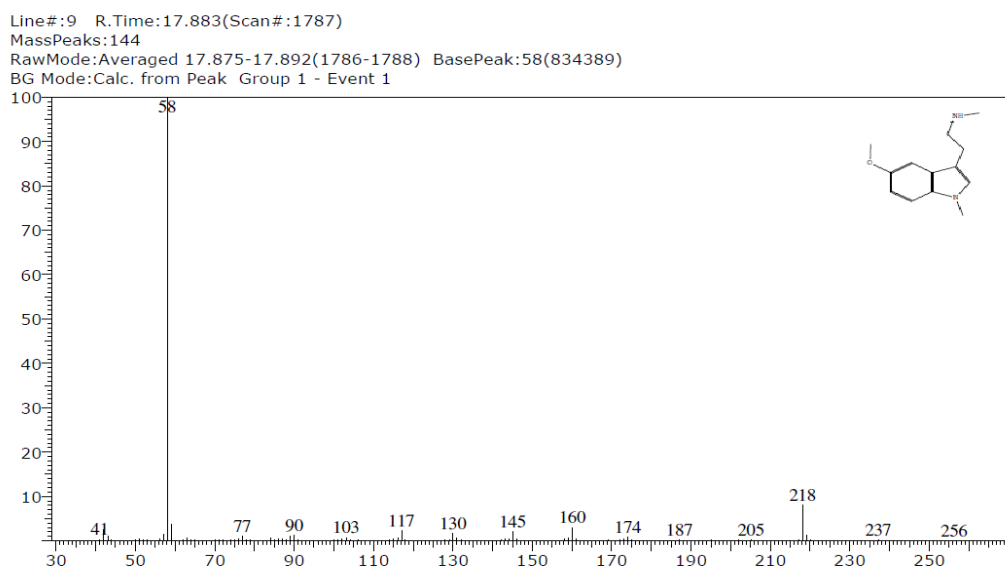


Figure.5. 5-hydroxyproline (RT: 9.328)



**Figure.6.(-)-Guaiol (RT: 13.422)**



**Figure.7. 1H-Indole-3-Ethanamine - 5-Methoxy-N,1-Dimethyl (RT: 17.882)**

## CONCLUSION

In the present study twenty five chemical constituents have been identified from ethanolic extract of the root of *Glycosmis pentaphylla* by Gas chromatography- Mass spectrometry (GC-MS) analysis of which six compounds have not been reported in previous studies. The presence of various bioactive compounds justifies the use of the root for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Glycosmis pentaphylla* contains various bioactive compounds. So it is recommended as a

plant of phytochemical importance. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and Pharmacological products.

## REFERENCES

1. Anonymous, Wealth of India: A Dictionary of Raw material and Industrial products, Vol. IV, Publication and Information Directorate, CSIR, New Delhi, 150-151, (2003).
2. Kiruthikar K R. and Basu B D, Ed. Indian Medicinal Plants, Vol I, M/S Bishen Singh, Mahendra Palsingh, New Delhi: 469-471, (1996).
3. K.M. Nadkarni, Ed. Indian Material Medica, Vol 1, Popular Prakashan Publishers: 580-581 (1954).
4. McKenzie A.W, Prince J.R, Alkaloids of the Australian rutaceae: *Glycosmis pentaphylla* (Retz.) Correa. Aust.J.Sci.Res. A 5 : 580 (1952).
5. Chakraborty D.P, Glycozoline, a carbazole derivative, from *Glycosmis pentaphylla* (Retz.) DC. Phytochemistry, 8: 769-772, (1969).
6. Sarkar M, Chakraborty D.P, Glycophymoline, a new minor quinazoline alkaloid from *Glycosmis pentaphylla* (Retz.) DC. Phytochemistry, 18: 694-695, (1979).
7. Mukherjee S, Mukherjee M, Ganguly S.N, Glycozolinine, a carbazole derivative from *Glycosmis pentaphylla* (Retz.) DC, Phytochemistry, 22: 1064-1065, (1983).
8. Bhattacharyya P, Chakrabarty P.K, Chowdhury B.K, Glycozolidol, an antibacterial carbazole alkaloid from *Glycosmis pentaphylla* (Retz.) DC, Phytochemistry, 24(4): 882-883, (1985).
9. Gopalakrishnan S, Vadivel E, GC-MS analysis of some bioactive constituents of *Mussaenda frondosa* Linn. International Journal of Pharma and Biosciences, 2(1): 313-320, (2011).
10. Lacikova L, Zapletal J, Masterrova I, Grancai D, GC-MS analysis of leaves of petrol ether extracts from four *Staphylea* L. Species. Acta Facult Pharm Univ Comenianae, 54: 104-108, (2007).
11. P. K. Warrier, V. P. K. Nambiar, C. Ramankutty, Indian Medicinal Plants, Vol 3, Orient Longman Publishers: 82-83, (1995).