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Identification of Active Phytocomponents in the Ethyl Acetate Extract of *Glycosmis pentaphylla* Retz. DC by Using GC-MS

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Abstract*—Glycosmis pentaphylla* is one of the medicinally important plants belonging to the family *Rutaceae*, commonly known as "Anam or Panal" in Tamil. Traditionally, leaves are useful in fever, hepatopathy, eczema, skin disease, helminthiasis, wounds, and erysipelas. The fruits are sweet and are useful in vitiated conditions of vata, kapha, cough, and bronchitis. The roots are good for facial inflammations, rheumatism, jaundice, and anemia. The preliminary phytochemical investigations indicated the presence of alkaloids, terpenoids, flavonoids, tannins, sugar, glycoside, and phenolic compounds. In the present study, the root part of *Glycosmis pentaphylla* was used, and the root was collected from Western Ghats of South India. The root was sun/shade dried and pulverized to powder in a mechanical grinder. The powder was successively extracted with various solvents, and the ethyl acetate extract of *Glycosmis pentaphylla* has been subjected to the GC-MS analysis. Amongst the 46 chemical constituents identified from this plant, three major phytoconstituents were reported for the first time. Marmesin, a furanocumarin compound with the chemical structure 7H-Furo (3,2-G) (1)Benzopyran-7-one,2,3–dihydro–2 - (1-Hydroxy-1methylethyl)-(s) is one of the three compounds identified for the first time at the concentration of 11-60% in ethyl acetate extract of *Glycosmis pentaphylla*. Others include, Beta.-Fagarine (4.71%) and Paverine (13.08%)

***Keywords*—**Ethyl acetate extract, *Glycosmis pentaphylla,* GC-MS analysis, phytochemicals.

I. Introduction

GLYCOSMIS pentaphylla Retz. DC (*Rutaceae*) commonly known as orange berry and gin berry in a small tree or shrubs is distributed throughout India. The plant is known by various names in different languages as "Vananimbuka" in Sanskrit, "Ban-nimbu" in Hindi, "Anam" in Tamil, "Manikyan" in Kannada, "Panal" in Malayalam and "Gongi pandu" in Telgu [1]-[3]. The preliminary phytochemical screening identified the presence of alkaloids, terpenoids, flavonoids, tannins, sugar, glycoside, and phenolic compounds [4], [5]. The various parts of plants were traditionally used for various ailments. The roots are used in inflammation, rheumatism, jaundice, and anemia. Also, the plant is reported as astringent, vermifuge, febrifuge, and anti-inflammatory, helminthiasis, cough, bronchitis, rheumatism, jaundice, hepatopathy, eczema, skin diseases, wounds, erysipelas [6]. The aim of the present study is to investigate the phytochemical composition of the plant by GC-MS analysis.

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II. Materials and Methods

A. Collection of Plant Material

The root of *Glycosmis pentaphylla* was collected in the months of January from Tirumala hills, Tirupathi, Western Ghats of South India, Andhara Pradesh. The plant was identified by Prof. P. Jayaraman, plant anatomy research center, West Thambaram, Chennai, Tamil Nadu, India. Avoucher specimen (SRU/FOP/2013/18) has been kept in the herbarium of the department of Pharmacognosy, Faculty of pharmacy, Sri Ramachandra University, Porur, Chennai-600 116, Tamil Nadu, India.

B. Preparation of Powder and Extract

By using a mechanical grinder, the root was shade dried and pulverized to powder. The powder (1.5 kg) was successively extracted with various solvents such as petroleum ether (40-60 °C), chloroform, ethyl acetate, ethanol, and water. The extracts were concentrated under reduced pressure in a rotary evaporator (Technico, India) [7]. The ethyl acetate extracts of the root of *Glycosmis pentaphylla* was used for GC-MS analysis.

C. GC-MS Analysis-Preparation of Extracts

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

D. Instruments and Chromatographic Conditions

GC clarus 500 Perkin Elmer system was used to carry out the GC-MS analysis of our prepared ethyl acetate extract of *Glycosmis pentaphylla* which comprises a AOC-20i autosampler and a gas chromatograph connected to a mass spectrometer (GC-MS). The instrument was equipped with an elite – 1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane) which is operated in electron impact mode at 70 eV. The carrier gas used was helium (99.999%) with constant flow of 1ml/min, and the volume of injection employed was 0.5 EI (split ratio of 10:1). The temperature of injector and the ion-source temperature were fixed as 250 °C & 280 °C, respectively. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to 200 °C/min, then 5 °C/min to 280 °C/min, ending with a 9 min isothermal at

280 °C. The spectra were taken at 70 eV with a scan interval of 0.5 s, and fragments were taken from 40 to 550 Da [8]-[10].

III. Results and Discussion

The phytochemical screening of ethyl acetate extract of the root of *Glycosmis pentaphylla* has shown the presence of alkaloids, phenols, flavonoids, glycosides, tannins, terpenoids and carbohydrate. A further identification of the phytocomponents has done by using GC-MS analysis.

A. Identification of Components

The GC-MS analysis of ethyl acetate extract of *Glycosmis pentaphylla* was done, and interpretations of the results were correlated with the database of (NIST) National Institute Standard and Technology. The spectrum of the unknown constituents present in ethyl acetate extract of *Glycosmis pentaphylla* was compared with the spectrum of the known constituents listed in the NIST library [11]. The results were interpreted and displayed in Table I.

TABLE I
Identified Phytocomponents in the Ethyl Acetate Extract of the Root of *Glycosmis pentaphylla* by GC-MS

PN	RT	PAP	Name of the Compound	MF	MW
1	6.105	2.60	1,2,3-Propanetriol monoacetate	$C_5H_{10}O_4$	134
2	6.515	1.39	3,5,5-Trimethyl-2-cyclohexen-1-one	$C_9H_{14}O$	138
3	8.466	2.62	2,3-dihydroxypropyl acetate	$C_5H_{10}O_4$	134
4	9.741	0.31	Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3r-trans)-	$C_{15}H_{24}$	204
5	10.331	0.26	Copaene	$C_{15}H_{24}$	204
6	10.476	0.52	1-Tetradecene	$C_{14}H_{28}$	196
7	10.512	0.52	2,4-Diisopropenyl-1-Methyl-1-Vinylcyclohexane	$C_{15}H_{24}$	204
8	10.951	1.32	Caryophyllene	$C_{15}H_{24}$	204
9	11.037	0.34	Naphthalene,	$C_{15}H_{24}$	204
10	11.433	1.99	Alpha.-caryophyllene	$C_{15}H_{24}$	204
11	12.092	1.15	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206
12	12.132	0.34	Carbamic acid, methyl-, o-cumenyl ester	$C_{11}H_{15}NO_2$	193
13	12.612	2.20	2-(4,8-Dimethyl-3,7-cyclodecadien-1-yl)-2-propanol	$C_{15}H_{26}O$	222
14	12.809	1.44	Canophyllal	$C_{30}H_{48}O_2$	440
15	13.015	0.90	1-Heptadecene	$C_{16}H_{32}$	224
16	13.203	0.92	Guaiol	$C_{15}H_{26}O$	222
17	13.354	2.57	Rosifoliol	$C_{15}H_{26}O$	222
18	13.424	4.25	Champaca camphor	$C_{15}H_{26}O$	222
19	13.895	2.16	Gamma.-Eudesmol	$C_{15}H_{26}O$	222
20	13.943	1.14	Epiglobulol	$C_{15}H_{26}O$	222
21	14.141	2.69	Guai-1(5)-en-11-ol	$C_{15}H_{26}O$	222
22	15.266	1.11	1,2-Tetradecene	$C_{19}H_{38}$	266
23	16.074	1.00	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278
24	17.023	1.60	Dictamnine	$C_{12}H_9NO_2$	199
25	17.300	2.33	1-Nonadecene	$C_{19}H_{38}$	266
26	18.365	1.46	8,8-Dimethyl-2H,8H-pyrano[3,2-g]chromen-2-one	$C_{14}H_{12}O_3$	228
27	19.153	1.33	n-Tetracosanol-1	$C_{24}H_{50}O$	354
28	19.309	0.62	2-Isopropenyl-2,3-dihydrofuro[3,2-g]chromen-7-one	$C_{14}H_{12}O_3$	228
29	19.590	3.78	Cyclopenta[c][1]benzopyran-4(1H)-one, 7-(dimethylamino)-2,3-dihydro	$C_{14}H_{15}NO_2$	229
30	20.092	0.98	Tetralin, 6-acetyl-8-isopropyl-2,5-dimethyl-	$C_{17}H_{24}O$	244
31	20.572	11.60	7h-Furo[3,2-g][1]benzopyran-7-one, 2,3-dihydro-2-(1-hydroxy-1-methylethyl)-, (s)-	$C_{14}H_{14}O_4$	246
32	20.837	4.71	Beta.-Fagarine	$C_{14}H_{13}NO_4$	259
33	21.270	0.81	1-Methoxybenzene,-4-(2-hydroxybenzylideneamino)	$C_{14}H_{13}NO_2$	227
34	21.683	2.48	Tetrapentacontan, 1,54-Dibromo-	$C_{54}H_{108}Br_2$	914
35	22.025	4.38	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	278
36	23.901	2.23	Tetratetracontane	$C_{24}H_{38}O_4$	390
37	24.023	0.59	Squalene	$C_{29}H_{60}$	408
38	24.590	1.87	Hexatriacontane	$C_{36}H_{74}$	408
39	25.257	1.97	Tetratetracontane	$C_{30}H_{50}$	410
40	25.814	1.53	N-Demethylacronycine	$C_{19}H_{17}NO_3$	307
41	25.903	2.02	N-Tetracontane	$C_{40}H_{82}$	562
42	25.970	4.38	6-(P-Tert-Butylphenoxy)-1,3-Dihydro-1,3-Diiminoisoindole	$C_{18}H_{19}N_3O$	293
43	26.565	13.08	Paverine	$C_{20}H_{21}NO_2$	307
44	27.237	0.76	Stigmasterol	$C_{29}H_{48}O$	412
45	27.336	0.92	n-Tetracontane	$C_{36}H_{74}$	506
46	27.770	0.87	gamma.-Sitosterol	$C_{29}H_{50}O$	414

PN- Peak number, RT- Retention Time, PAP - Peak area Percentage, MF - Molecular Formula, MW - Molecular Weight

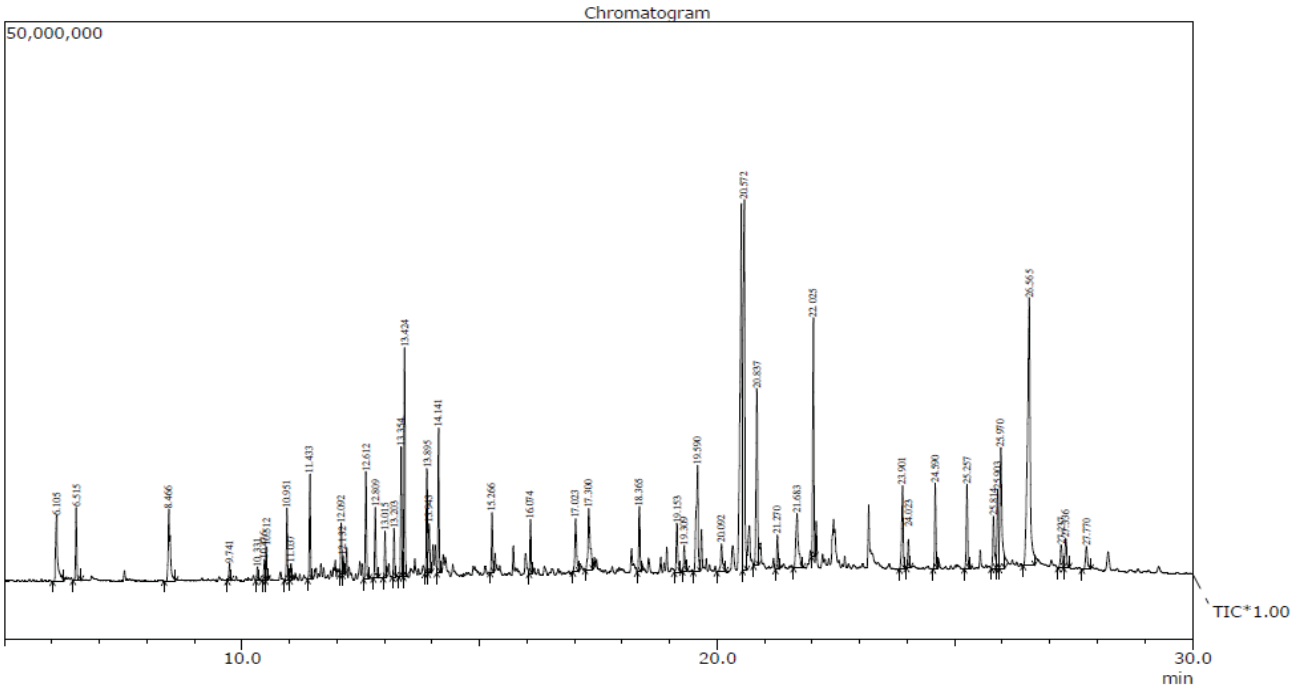

Fig. 1 GC-MS Chromatogram of ethyl acetate extract of the root of *Glycosmis pentaphylla*

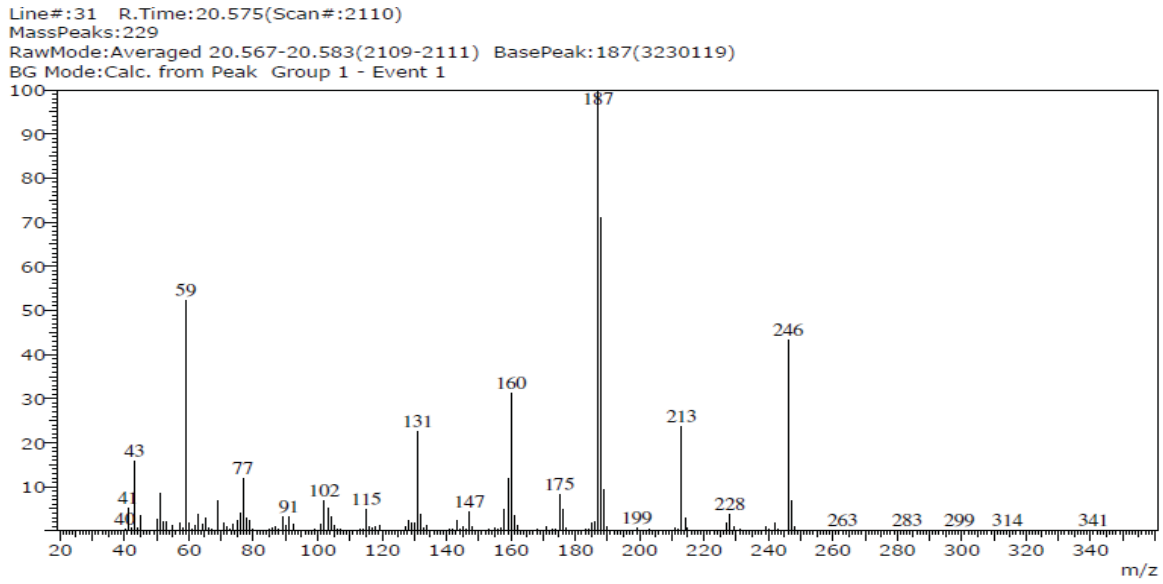

Fig. 2 7H-Furo(3,2-G)(1)Benzopyran-7-one,2,3-dihydro-2-(1-Hydroxy-1methylethyl)-,(s)- (RT: 20.510)

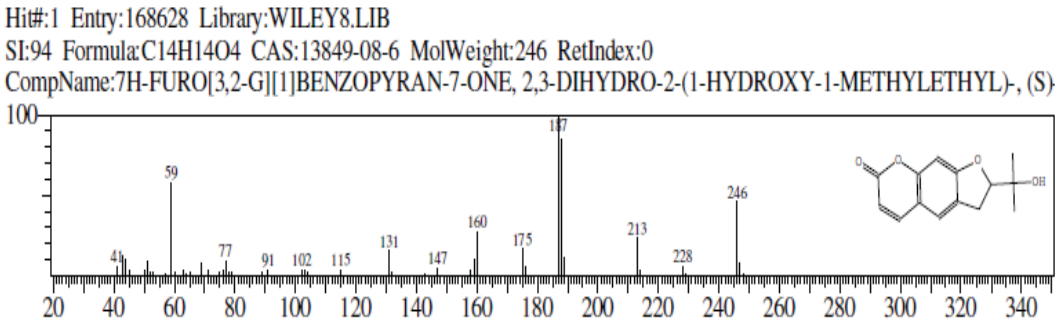

Fig. 3 7H-Furo(3,2-G)(1)Benzopyran-7-one,2,3-dihydro-2-(1-Hydroxy-1methylethyl)-,(s)- (RT: 20.510) with chemical structure

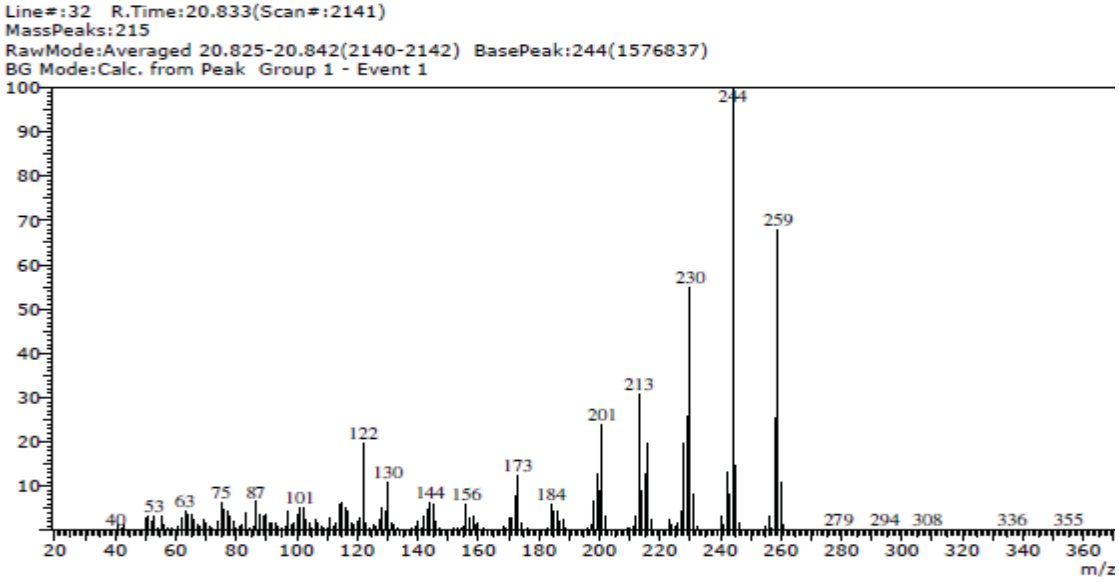

Fig. 4 Beta.-Fagarine

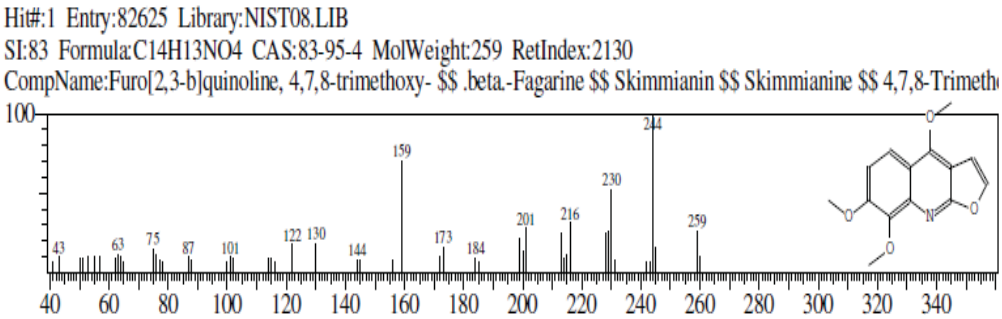

Fig. 5 Beta. -Fagarine fragmentation with chemical structure

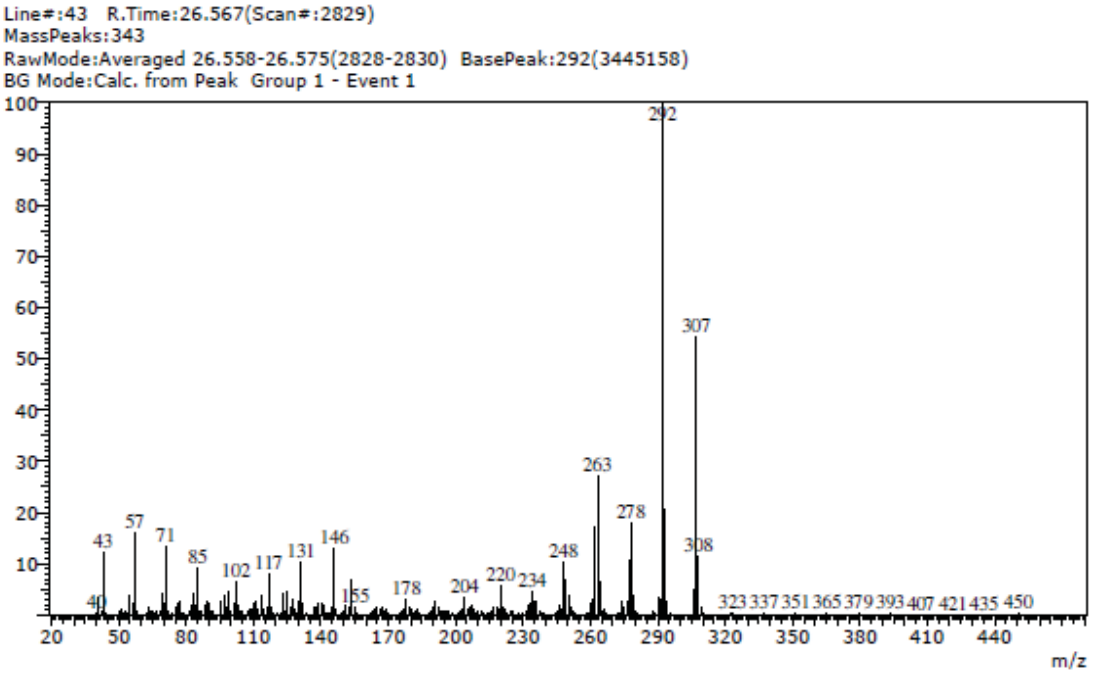

Fig. 6 Paverine

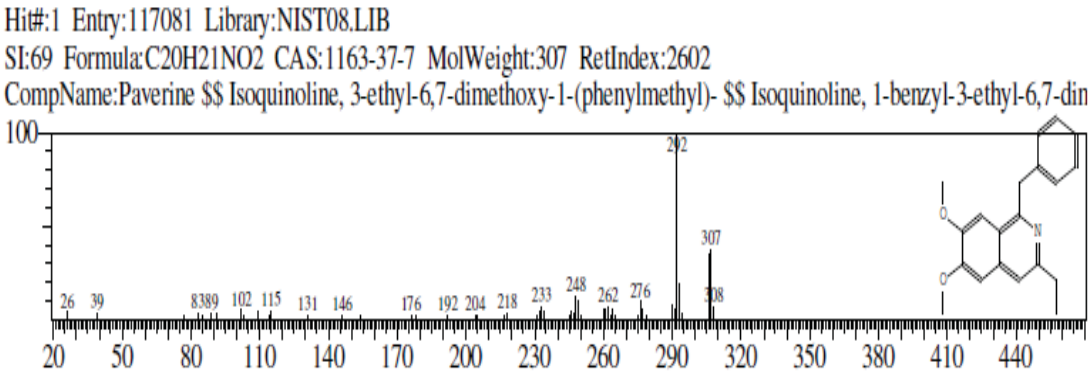

Fig. 7 Paverine fragmentation with chemical structure

IV. Conclusion

The GC-MS chromatogram of the ethyl acetate extract of root of *Glycosmis pentaphylla* revealed the presence of 46 compounds. Among the various phyto compounds identified, Marmesin, furanocumarin compounds 7HFuro (3,2-G) (1) Benzopyran-7 one,2,3 – dihydro – 2 - (1-Hydroxy-1methylethyl)-(s)-(11.60%) which are also called as compounds, Beta.-Fagarine (4.71%) and Paverine (13.08%) were found to be the major constituents. The present study which explored the phytochemical composition of the root of *Glycosmis pentaphylla* also provides justification for its traditional use against various diseases. Isolation of lead molecule responsible for its therapeutic efficacy should be undertaken to provide a new natural compound to the world seeking for the remedy with less or without side effects.

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