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Phytochemical screening, GC-MS analysis of *Decalepis hamiltonii* Wight & Arn. An endangered medicinal plant

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Abstract
The present paper reports the phytochemical and GC-MS analysis studies of an endemic endangered, climbing shrub. *Decalepis hamiltonii*. *D. hamiltonii* which belongs to the family Asclepiadaceae is a perennial slow growing medicinal shrub commonly called as "Maredu kommulu, Nannari kommulu". It is generally considered as a tubers root food mostly in the southern part of India. There is a growing demand for roots of *D. hamiltonii* in the pharmaceutical trade due to its use as a anti-inflammatory, degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, neurodegenerative diseases and others. In the present study the qualitative and analysis confirmed the presence of various phytochemicals like alkaloids, flavonoids, phenols, steroids and terpenoids. Quantitative estimation of flavonoids and phenols was also carried out and further analysis of the components present in it by GC-MS analysis. The roots were sequentially extracted by methanol. The extract showed the presence of all phytoconstituents studied. The GC-MS analysis of the methanolic extract revealed the presence of ten major compounds. This study forms a basis for the biological characterization and importance of the compounds identified.

Keywords: Phytochemical analysis, root extract, qualitative and quantitative analysis, GC-MS, *Decalepis hamiltonii*

1. Introduction
Indian traditional system of medicine is based on various systems of medicine such as Ayurveda, Siddha, Unani and Homoeopathy. During the last few years the graph of standardization of medicinal plants of potential therapeutic significance has been increased. The evaluation of all medicinal plants is based on phytochemical and pharmacological approaches which lead to drug discovery and it is referred to as "natural product screening" [1]. Secondary products from the plants are responsible for its action or pharmacological activity [2, 3]. *Decalepis hamiltonii* Wight & Arn. The species is endemic and endangered to peninsular India geographical distribution of *D. hamiltoni* is in southern India and rare in evergreen forests of Western Ghats and commonly called as maredu kommulu or barre sugandhi or maradu gaddalu or makali beru belonging to the family Asclepediaceae [4, 5]. It has been recorded in the dry and moist deciduous forests of Karnataka, Andhra Pradesh and Tamil Nadu. Its roots have been used in Ayurveda, the ancient Indian traditional systems of medicine to stimulate appetite, skin diseases, diaphoretic, hemorrhoids, rheumatism, asthma, bronchitis, diaphoretic, somatic and antiviral and as a general tonic [6]. It is also useful as a blood purifier, preservative, diarrhea, respiratory disorders, fever, bronchitis, asthma, eye diseases, urinary disorders, loss of appetite, burning sensation and rheumatism and especially for epileptic fits in children and as a source of bio insecticide for stored food grains [7]. Earlier studies have shown that roots contains aldehyde, inositols, amyrins and lupeols [8] as well as volatile compounds such as 2-hydroxy- 4-methoxy benzaldehyde, vanillin, 2-phenylethyl alcohol, benzaldehyde and others [9]. The plant has a use in many medicinal preparations and due to this there is a heavy demand for it. As the whole plant is uprooted from its natural habitat for the use of the herbal drug industry its numbers are decreasing drastically in the natural population. To meet the huge demand for its supply, there is a need to develop a specific technology for production of *D. hamiltonii* in a large scale. The maintenance of genetic purity is a limitation for large scale cultivation. *Decalepis* is one of the most important medicinal and economical properties or related information of all the species of *Decalepis* over its range of distribution, current status and the role of biotechnology in the conservation of this important genus. The roots of *D. hamiltonii* are little bitter and then sweet.

It is so characteristic with a familiar lingering after taste and smell of vanillin, the substance that is in *Vanilla planifolia*, an orchid used in ice-creams, chocolates, drinks etc. Although vanillin has been synthesized since 1874 natural source of this flavoring are still in demand and the roots of Decalepis species can be used as substitute for vanillin [10]. The present study of GC-MS analysis and phytochemical investigation of an endangered medicinal plant *D. hamiltonii* has been taken up to carry out gas chromatography and mass spectra analysis of root extract and qualitative phytochemical analysis for alkaloids, flavonoids, tannins, saponins, phenols, steroids, terpenoids and glycosides present in roots, stem and leaves.

Materials and methods

Material

Decalepis hamiltonii plants collected from Botanical garden Department of Botany, University College of Science, Saifabad, *D. hamiltonii*. is a slow growing, perennial woody climber of tropical and subtropical regions with a twining woody stem and opposite petiolate leaves, entire, smooth shiny, varying in shape. Flowers are small, in axillary sessile racemes. The root is long, rigid and cylindrical. These plants were subjected to phytochemical investigation studies (Qualitative and Quantitative) and GC-MS analysis and for the presence of important secondary metabolite compounds.

Preparation of extracts

Plant samples root, leaves and stem were washed with distilled water and air-dried at room temperature for 7-10 days, then oven-dried at 40 ^{0}C to remove the residual moisture. The dried plant parts were pulverized and stored in air-tight containers at 4 ^{0}C for future use. 50 g of powdered samples of gum were extracted with methanol by soxhlation method at 60 to 80 ^{0}C. The three filtrates were separately concentrated in water bath at 40 ^{0}C and evaporated under reduced pressure.

Phytochemical analysis

The extracts obtained from the powdered root, leaves and stem of *D. hamiltonii* were subjected to phytochemical tests to determine the presence of active secondary metabolites using standard procedures. This extract was filtered through a fine mesh into a test tube. This crude extract was used for the phytochemical investigation of secondary metabolites, GS-MS tests given below and the tests were carried out in triplicate.

Qualitative analysis

Test for identification of Alkaloids

About 0.5 gm of methanol extract was taken in a test tube and was diluted and homogenized with 10 ml distilled water, dissolved in 20 ml dilute HCl solution and clarified by filtration. The filtrate was tested with Dragendorff's and Mayer's reagent. The treated solution was observed for precipitation of white or creamy colour.

Test for identification of Flavonoids

About 0.5 gm of extract was introduced into 10 ml of ethyl acetate in a test tube and heated in boiling water for 1 min. The mixture was then filtered. About 4 ml of the filtrate was shaken with 1 ml 1% aluminium chloride solution and incubated for 10 min. Formation of yellow colour in the presence of 1 ml dilute ammonia solution indicated the presence of flavonoids.

Test for identification of Phenols

About 0.5 gm of extract was taken in a test tube, mixed with 100ml distilled water and heated gently. To this, 2 ml of ferric chloride solution was added and observed for the formation of green or blue colour.

Test for identification of Saponins

About 0.5 gm of methanol extract was taken in a test tube and 5 ml distilled water was added to it. The solution was shaken vigorously and observed for persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for identification of Steroids

About 0.5 gm of methanol extract was taken in a test tube and 2 ml of acetic anhydride was added to it and 2 ml of sulphuric acid was added by the sides of the test tube and observed for the colour change to violet or blue green.

Test for identification of Tannins

Five grams of the ground powder was extracted with 10 ml ammoniacal chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M sulphuric acid. Creamish white precipitate was observed for the presence of tannins.

Test for identification of Terpenoids

5 ml of the methanol extract was mixed with 2 ml of chloroform and 2ml concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of Terpenoids.

Quantitative analysis

Quantitative analysis was carried out to estimate total flavonoids and total phenols.

Determination of total flavonoids

Aluminium chloride - colorimetric method [11] with some modifications was used to determine flavonoid content. 1.0 ml root extract was mixed with 1.0 ml methanol, 0.5 ml aluminium chloride (1.2%) and 0.5 ml potassium acetate (0.1176%). The mixture was allowed to stand for 30 min at room temperature. Later the absorbance was measured at 415 nm. Quercetin was used as standard. Flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

Determination of total phenols

Total phenolic content of the extracts was determined by Folin Ciocalteu reagent method [12] with some modifications. The root extract (1.0 ml) was mixed with Ciocalteu reagent and allowed to stand for 15 min and 5 ml of saturated Na_2CO_3 was added. The mixture was allowed to stand for 30 min at room temperature and the total phenols were determined spectrophotometrically at 760 nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of gallic acid equivalent (mg/ g of extracted compound).

GC-MS Analysis

GC-MS analyses of methanol extract were performed using a Shimadzu QP2010 Gas-Chromatography–Mass spectroscopy. It employed a fused silica column packed with Elite -5 ms [5% Diphenyl 95% Dimethyl poly siloxane, 30 mm × 0.25 mm × 0.25 µm df] and the components were separated using

helium as carrier gas at a constant flow of 1ml / min. The 2 µl sample extract injected in to the instrument. It was detected by the turbo gold mass detector with aid of Turbo mass 5.2 software. During the GC Process the oven was maintained at temperature of 110 ^{0}C with 2 min holding. The injector temperature was set at 250 ^{0}C. The inlet line temperature was 200 ^{0}C and source temperature was 200 ^{0}C. Mass spectra were taken at 70 eV, a scan period of 0.5 S and fragment from 45 - 450 Da. The MS detection was completed in 36 min. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute standard and technology (NIST and WILEY) having more than 62,000 patterns. The spectrum of unknown components stored in the NIST and WILEY library.

Results and discussion

The present study contributes valuable information of bioactive compounds in *Decalepis hamiltonii*. Qualitative analysis of plant extract was carried out for Alkaloids, Flavonoids, Phenols, Steroids, Tannins, Terpenoids, Saponins and Glycosides. All of the phytochemicals like Alkaloids, Flavonoids, Phenols, Steroids, Tannins, Terpenoids, Saponins and Glycosides were present in root extract and Alkaloids, Flavonoids, Phenols, Steroids and Terpenoids were present in leaves and stem extract of *D. hamiltonii* except Saponins, Glycosides and Tannins (Fig-3, Table-1) which is similar to the reports of Asclepiadaceae family [13, 14, 15]. The plant extracts were quantitatively analyzed for Flavonoids and Phenol (Table-2). Whereas, our study reports the absence of Saponins [16]. Indicated that Saponins were present in *D. hamiltonii* in the methanol extract. Several medicinal properties have been attributed to Saponins [16] but surprisingly, Saponins were not found in the present study. Flavonoids and Phenol are however reported in the present study which agrees with the findings of [16, 17] who has attributed anti-diabetic, anti-aging anti-inflammation and bactericidal effects.

Gas chromatography coupled mass spectrometry (GC-MS) is an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. The GC-MS analysis of methanol extracts was performed using a Shimadzu QP-2010 Gas- Chromatography –Mass spectroscopy. Analysis on GCMS was carried out with reference to NIST and WILEY library at Central analytical facility University College of Technology, Osmania University, Hyderabad, containing more than 62000 patterns. The spectra of unknown compounds were compared with spectra of known compounds stored in identification of compounds was confirmed based on the active principle, Molecular Weight (MW), Concentration (%), Retention Time (RT), Molecular Formula (MF) and Peak Area (PA) is presented in (Fig-4 and Table-3). More than ten major compounds were identified in the extract being Furfural, Methyl-2-Furoate, 2-hydroxy-4-methoxy benzaldehyde, Vanillin, Tetradecane, Diethyl Phtalate, Hexadecane, Carbromal, Lupeol, Norolean-12-En respectively along with other minor constituents. The identified compounds in the roots of methanolic extract of *D. hamiltonii*. These similar studies was conducted on different parts of *D. hamiltoni* [13, 18]. The identified compounds in the roots of methanolic extract of *Decalepis hamiltonii* possess many biological properties. Among the identified phytochemicals, phenolic compound have the property of antioxidant and antifungal activity study as situation has forced to search new antimicrobial substances in various sources like medicinal plants [19].

Fig 3: Test results of qualitative analysis of phytochemical constituents of the methanol extract of *D. hamiltoni*
A: Test results roots extract, B: Test results stem extract and C: Test results leaves extract.

Table 1: Qualitative analysis of phytochemical constituents of the methanol extract of roots, leaves and stem from *D. hamiltoni.*

S. No	Test for Phytochemicals	Test results		
		Root	Leaves	Stem
1	Alkaloids	+*ve*	+*ve*	+*ve*
2	Flavonoids	+*ve*	+*ve*	+*ve*
3	Phenols	+*ve*	+*ve*	+*ve*
4	Steroids	+*ve*	+*ve*	+*ve*
5	Tannins	+*ve*	-*ve*	-*ve*
6	Terpenoids	+*ve*	+*ve*	+*ve*
7	Saponins	+*ve*	-*ve*	-*ve*
8	Glycosides	+*ve*	-*ve*	-*ve*

+ *ve* Presence of the compound.
- *ve* Absence of the compound.

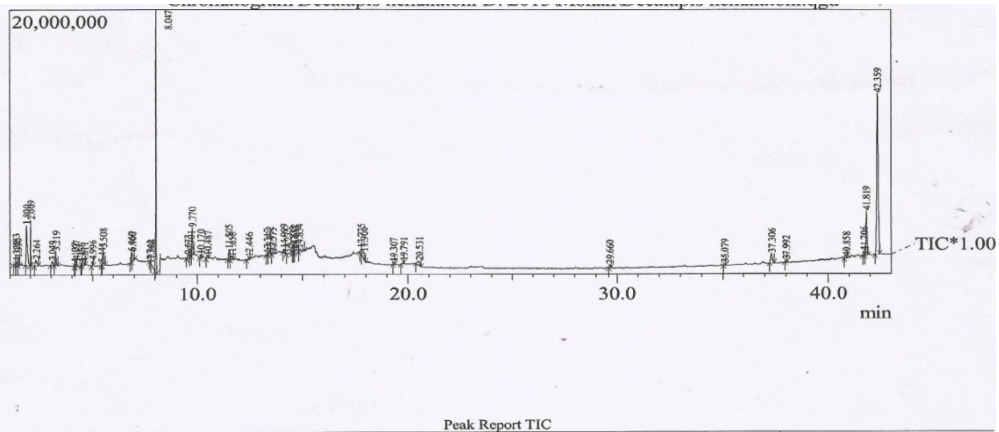
Table 2: Quantitative analysis of the methonol root extracts of *D. hamiltoni* for estimation of Flavonoids and Phenols.

S. No	Plant extract	Phytochemicals	Average Estimated value (mg/gm) (Mean±S.E)
1.	Flavonoids	Root	7.95±0.85
2.	Phenols	Root	14.22±0.96

* Phenols are expressed as Gallic acid equivalent (GAE) and Flavonoids are expressed as Quercetin equivalents (QE) in mg/100 gm.

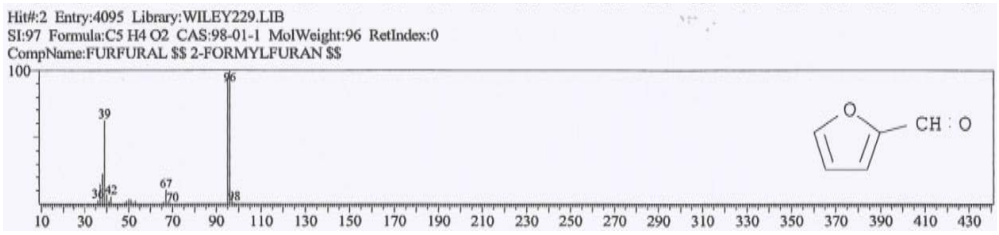
Table 3: Components detected in the root of methanol extract of *Decalepis hamiltonii*.

S. No	RT	Name of the component	Molecular formula	MW	Peak of Area %
1	1.800	Furfural	$C_5H_4O_2$	96	6.47
2	4.611	Methyl-2-Furoate	$C_6H_6O_2$	110	0.22
3	8.047	2-hydroxy-4-methoxy benzaldehyde	$C_8H_8O_3$	152	21.48
4	9.770	Vanillin	$C_8H_8O_3$	152	2.68
5	10.487	Tetradecane	$C_{14}H_{30}$	198	0.36
6	11.505	Diethyl Phtalate	$C_{12}H_{14}O_4$	222	1.18
7	11.658	Hexadecane	$C_{16}H_{34}$	226	0.59
8	40.858	Carbromal	$C_7H_{13}BrN_2O_2$	236	0.53
9	41.706	Lupeol	$C_{32}H_{52}O_2$	468	0.56
10	42.359	Norolean-12-Ene	$C_{29}H_{48}$	396	29.23



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	1.183	1.167	1.300	582364	0.29	68638	0.10	8.48	1-Vinylimidazole
2	1.325	1.300	1.375	1034567	0.52	308027	0.46	3.36	1-Butanol, 2-nitro- (CAS) 2-Nitr
3	1.390	1.375	1.467	1860720	0.93	520974	0.77	3.57	2-Propanone, 1-hydroxy- (CAS)
4	1.800	1.750	1.983	12914484	6.47	3024784	4.48	4.27	Furfural
5	2.009	1.983	2.042	3545759	1.78	3379801	5.00	1.05	Benzeneacetic acid, .alpha.-[(trin
6	2.264	2.183	2.383	2889633	1.45	485942	0.72	5.95	Butanoic acid, 2-ethyl-, methyl e
7	3.049	2.967	3.133	1455608	0.73	345294	0.51	4.22	Pyrazine, methoxy-
8	3.219	3.133	3.325	2853013	1.43	1076928	1.59	2.65	4-HYDROXY-2,5-DIMETHYL
9	4.127	4.108	4.167	577473	0.29	294602	0.44	1.96	Pentanoic acid, 4-oxo-
10	4.197	4.167	4.242	870755	0.44	532560	0.79	1.64	1-(4-Hydroxy-3-methoxyphenyl)
11	4.466	4.433	4.492	463901	0.23	189121	0.28	2.45	N,N'-Dimethylpiperazine $$ Pipe
12	4.611	4.492	4.658	437321	0.22	214058	0.32	2.04	METHYL-2-FUROATE $$
13	4.996	4.958	5.050	1021217	0.51	429216	0.64	2.38	2-Cyclohexen-1-one (CAS) 2-Cy
14	5.441	5.408	5.467	555766	0.28	233345	0.35	2.38	Butanoic acid, 2-methyl-3-oxo-,
15	5.508	5.467	5.608	3750019	1.88	1314000	1.95	2.85	2,3-DIHYDRO-3,5-DIHYDRO
16	6.869	6.800	6.883	2360353	1.18	736896	1.09	3.20	2-Furancarboxaldehyde, 5-(hydr
17	6.900	6.883	7.000	2983399	1.50	671377	0.99	4.44	1-FURYL-1-ETHOXY-ETHAN
18	7.742	7.725	7.783	439734	0.22	108737	0.16	4.04	Propanoic acid, 2-methyl-, 3-(ace
19	7.808	7.783	7.983	6607437	3.31	364790	0.54	18.11	Octane, 1-(ethenylthio)- $$
20	8.047	7.983	8.067	42858990	21.48	28116169	41.62	1.52	Benzaldehyde, 2-hydroxy-4-meth
21	9.573	9.483	9.633	1045608	0.52	219619	0.33	4.76	Tetracosane
22	9.701	9.633	9.733	1269770	0.64	433339	0.64	2.93	Dodecane, 2,6,11-trimethyl-
23	9.770	9.733	9.850	5348179	2.68	2099126	3.11	2.55	Vanillin
24	10.170	10.133	10.217	669338	0.34	260168	0.39	2.57	Butanoic acid, 2-ethyl-2-methyl-
25	10.487	10.425	10.533	718224	0.36	196743	0.29	3.65	Tetradecane
26	11.505	11.458	11.558	2348053	1.18	762732	1.13	3.08	DIETHYL PHTALATE $$
27	11.658	11.558	11.700	1174923	0.59	190892	0.28	6.15	Hexadecane (CAS) n-Hexadecan
28	12.446	12.333	12.500	638633	0.32	156173	0.23	4.09	Docosane $$ n-Docosane $$ Nor
29	13.317	13.267	13.358	459616	0.23	162560	0.24	2.83	Octadecane, 1-iodo- (CAS) N-O
30	13.432	13.358	13.475	999740	0.50	272340	0.40	3.67	ALLYL CAPROATE $$
31	13.575	13.525	13.667	600124	0.30	145082	0.21	4.14	Dodecane, 2,6,11-trimethyl-
32	14.099	14.058	14.167	827539	0.41	275577	0.41	3.00	Tricosane
33	14.275	14.233	14.508	1659929	0.83	114948	0.17	14.44	Heptadecane
34	14.533	14.508	14.550	470902	0.24	198558	0.29	2.37	Phosphonic acid, [1-(acetylamino
35	14.592	14.550	14.617	821165	0.41	281426	0.42	2.92	Androst-5-ene-3,17-diol, 4,4-dim
36	14.633	14.617	14.817	1968519	0.99	173400	0.26	11.35	Borazine, 2,4,6-triphenyl-1,3,5-tr
37	14.834	14.817	15.075	1655501	0.83	227860	0.34	7.27	4-.ALPHA.,20-DIMETHYL-3-.
38	17.775	17.717	17.792	1207931	0.61	444817	0.66	2.72	Borazine, 2,4,6-triphenyl-1,3,5-tr
39	17.900	17.792	17.983	3785702	1.90	534225	0.79	7.09	HEPTAMETHYL-PHENYL-CY
40	19.307	19.275	19.367	556192	0.28	175513	0.26	3.17	Dibutyl phthalate
41	19.791	19.658	19.825	670638	0.34	148866	0.22	4.50	Mequinol
42	20.531	20.383	20.642	2612687	1.31	333618	0.49	7.83	NONAMETHYL, PHENYL-, C
43	29.660	29.608	29.717	877129	0.44	284995	0.42	3.08	Phthalic acid, diisooctyl ester $$
44	35.079	35.042	35.142	481604	0.24	146305	0.22	3.29	Cyclohexane, 1-ethenyl-1-methyl
45	37.306	37.217	37.442	3735358	1.87	733669	1.09	5.09	Campesterol $$ Ergost-5-en-3-ol
46	37.992	37.942	38.042	424511	0.21	145900	0.22	2.91	Azulene, 1,2,3,4,5,6,7,8-octahyd
47	40.858	40.758	40.883	1058557	0.53	127529	0.19	8.30	Carbromal
48	41.706	41.650	41.742	1127317	0.56	322027	0.48	3.50	Lupeol $$ Lup-20(29)-en-3-ol, (
49	41.819	41.742	41.900	11928608	5.98	3349661	4.96	3.56	Viminalol $$ Urs-12-en-3-ol, (3.
50	42.359	42.233	42.475	58324579	29.23	12218048	18.09	4.77	NOROLEAN-12-ENE $$
				199529089	100.00	67550975	100.00		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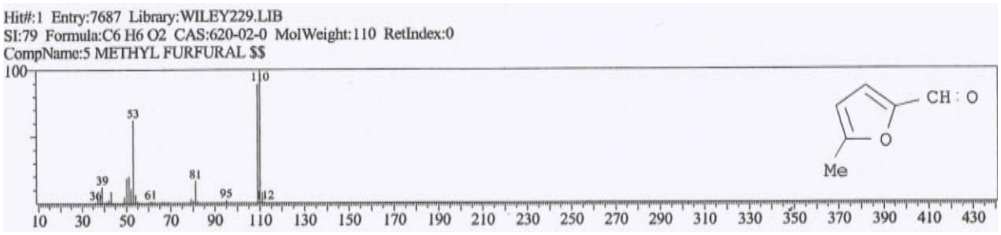
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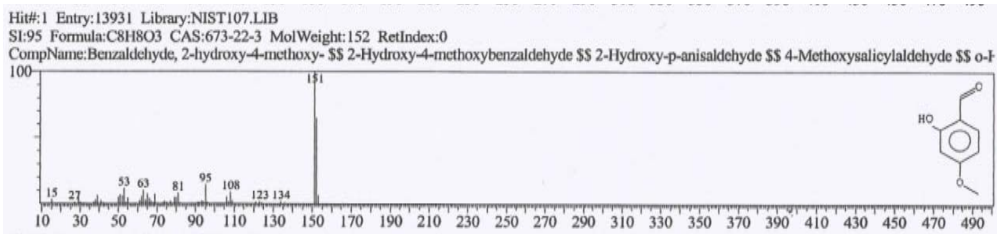
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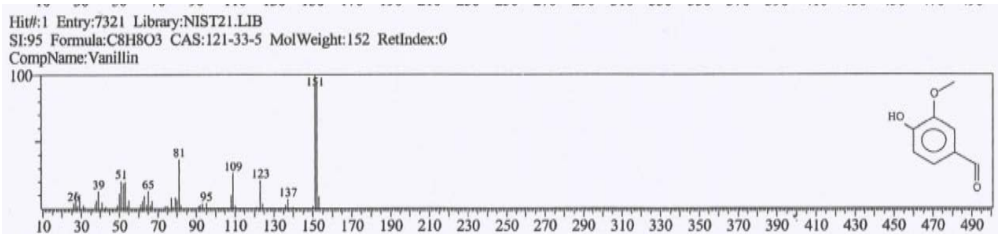
HO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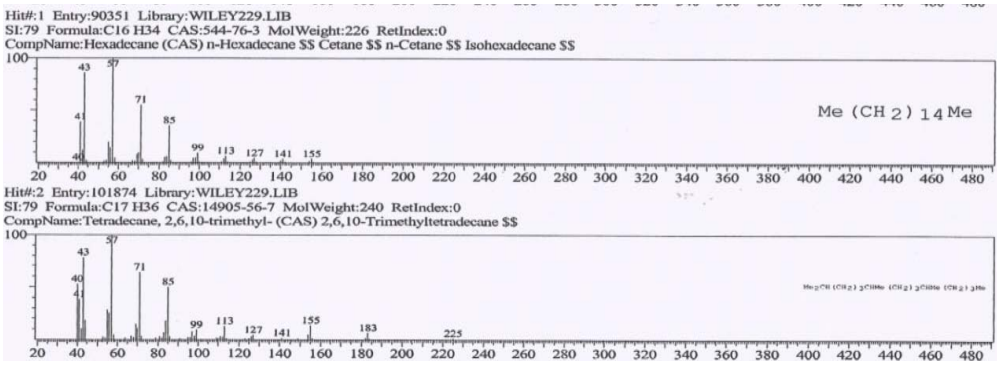
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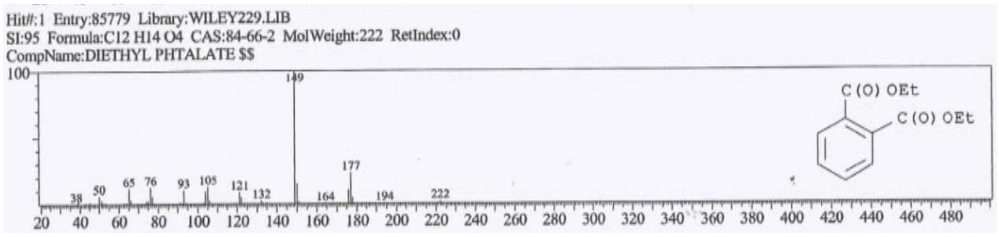
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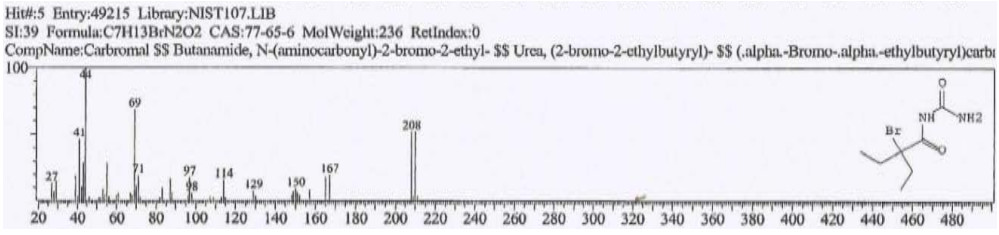
C (O) OEt
C (O) OEt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SI:39 Formula:C7H13BrN2O2 CAS:77-65-6 MolWeight:236 RetIndex:0
CompName:Carbromal $$ Butanamide, N-(aminocarbonyl)-2-bromo-2-ethyl- $$ Urea, (2-bromo-2-ethylbutyryl)- $$ (.alpha.-Bromo-.alpha.-ethylbutyryl)carb
NH
NH2
Br

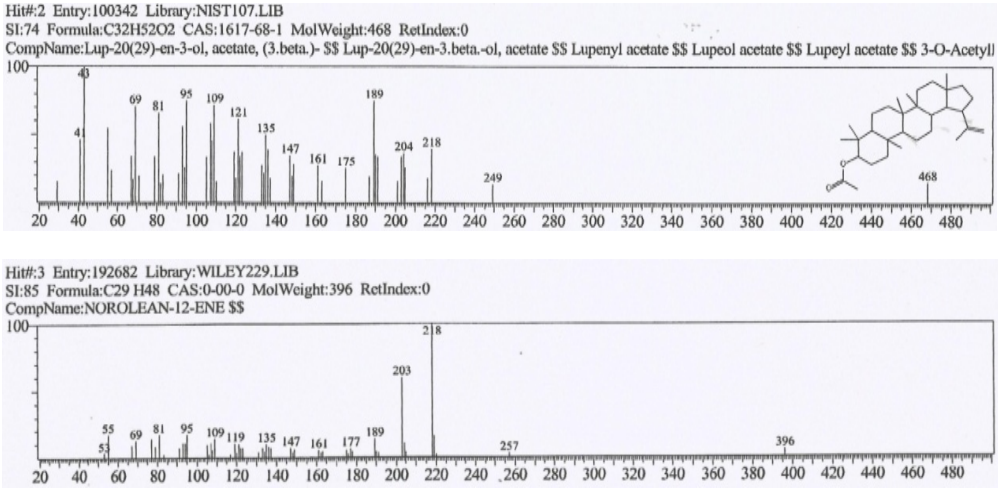

Fig 4: Mass spectrum showing of the methanol root extract of *Decalepis hamiltoni*.

Conclusion

It is concluded that *Decalepis hamiltonii* is a plant with a variety of ethnic medicinal uses. The qualitative and quantitative analysis of *Decalepis hamiltonii* shows the presence of bioactive compounds such as Alkaloids Flavonoids, Phenols, Tannins, Terpenoids and Glycosides. This is valuable information for preparation of drugs in pharmaceutical industry and stress the need for more intensive research since they play a great role in healthcare. The present study describes of GC-MS analysis of methanol extract of root of *D. hamiltonii* showed the presence of 10 bioactive components Furfural, Methyl-2-Furoate, 2-hydroxy-4-methoxy benzaldehyde, Vanillin, Tetradecane, Diethyl Phthalate, Hexadecane, Carbromal, Lupeol, Norolean-12-En. which suggests the contribution of these compounds on pharmacological activity. These active principles provide inspiration for further investigation in the discovery of novel herbal drugs. Hence, the roots, of *D. hamiltonii* might be utilized for the development of traditional medicines and further investigation is in need to elute novel active compounds which may create the new way to treat many incurable diseases.

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