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Metabolic profiling through GC-HRMS analysis of ethnomedicinal species *Pittosporum dasycaulon* MIQ. (Pittosporaceae)

Riyas Chakkinga Thodi, Swapna Thacheril Sukumaran
Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, India

Abstract
Medicinal plants are versatile sources of natural products with pharmacological importance, and therefore, it leads to interest in the researchers in pharmacology. The objective of this investigation was to determine the chemical constituents of the methanolic extract of leaves. The phytochemical compound was studied using GC-HRMS. Twenty important phytochemical compounds were identified based on the mass fragment, retention time, molecular weight in the methanolic extract of *Pittosporum dasycaulon* Miq. GC-HRMS analysis of *P. dasycaulon* indicated the presence of the 2 (3H)-Furanone dihydro-4-hydroxy; 2-Furanmethanol,5-ethenyltetrahydro-α,α,5-trimethyl-,cis-; D Alanine, N-propargyloxycarbonyl-isohexyl ester; 1,3,5-dihydroxy- 6-methyl-2-3-dihydro-4-pyran-4-one; Furandiol, tetrahydro-,cis; Benzoic acid, 2 butoxy, methyl ester; 2-Furancarboxaldehyde, 5 hydroxyl; 4-hydroxy 3 methyl acetophenone; 1-(3,6,6-trimethyl-1,6,7,7 a-terahydrocyclopenta (c) pyran-1-yl) ethanone; Sucrose; Alpha-1-rhamnopyranose, 3,7,11,15-tetramethyl –α-hexadecen-1 ol; (6z)-6-pentadecen-1-ol; n-hexadecanoic acid; Phytol; 7,10,13- hexadecatrienal; Octadecanoic acid; E-10,13,13, trimethyl-11-tetradecen-1-ol acetate; 1,3-hydroxyspirotene-en-11-one and Octadecane, 3 ethyl-5-(2-ethyl butyl). A GC-HRMS based method for metabolic profiling of the hydrophilic extract of *P. dasycaulon* was attempted for the first time.

Keywords: *Pittosporum dasycaulon*, GC-HRMS, phytochemicals, pharmacology

Introduction
Pittosporum dasycaulon Miq (Pittosporaceae) is an ethnomedicinal plant species endemic to moist deciduous evergreen forest above 800 m elevation. The *Pittosporum* is the only genus among the family Pittosporaceae found in India. The genus contains eleven species in India [1-2].

The different species of *Pittosporum* used traditionally used against various diseases, including cancer and inflammations [3]. The indigenous people endemic to south India have used *P. dasycaulon*, bark decoction against skin diseases, bronchitis, leprosy, and also used as antibacterial and antifungal remedy [4]. Remarkable anticancer compounds were also isolated from different species of *Pittosporum*, including Isosteviol (diterpene) and Olenolic acid derivative (trihydoxy olenolic acid) from the leaves of *P. tetraspermum*[5-6]. The cytotoxic compound, terpenoid glycoside (pittangretoside) from *P. angustifolium* [7], antiinflammatory guanine terpene from *P. undulatum* [8] were reported. Antioxidant and antiinflammatory potential of *P. viridiflorum* has also been reported in a previous study [9]. *P. dasycaulon* is an important medicinal source and is not yet received proper attention from the pharmacological field [4]. Although a few studies have been reported regarding the Phytoconstituents of *P. dasycaulon*, there is no report of gas chromatography and mass spectrometry (GC-MS) analysis. Therefore, the present study intended to analyze the phytoconstituents more extensively using GC-HRMS. This study will be helpful in the identification of secondary metabolites that can be an alternative method for the tracing of bioactive compounds.

Methodology
Collection and authentication of Plant material
The plant parts such as leaves and flowers of *P. dasycaulon* were collected from the southern part of Western Ghats (Wayanadu), Kerala, India, in 2019. The plant was identified and authenticated with the help of Flora (Figure 1). The leaves were washed thrice with distilled water to remove dust particles, shade dried for more than two weeks. The dried leaves were ground in an Electrical Mixture Grinder (BL Platinum 750 W- MG 139).

Fig 1: Showed a twig of the plant *Pittosporum dasycaulon* Miq. with young flower buds.

Soxhlet extraction method
The powdered crude extract leaves were stored in an air-tight container until further use. *P. dasycaulon* Miq leaves then dissolved in 300 mL of various organic solvents

(petroleum ether, chloroform, methanol, and water) and serially extracted using a soxhlet apparatus. After each extraction, the plant material was kept overnight for the evaporation of extraction solvent before the subsequent extraction with another solvent. Each dried extract were weighed to determine the percentage yield of each soluble constituent using the formula [10].

$$\% \ of \ yield = \frac{Weight \ of extract}{\text{Weigh of sample}} x100$$

The dried extracts of leaves were stored at 4◦C for further investigation, including GC-MS analysis.

GC-HRMS analysis.

Crude methanolic extract of *P. dasycaulon* leaves was used for GC-HRMS analysis [11]. The GC-MS analysis was carried out using a Agilent, 7890, FID detector, Head Space injector Combipal autosampler. Column temperature: initial temperature120 for 3 min. Ramp: 80C/min to 2700C. Again isothermal for 3 min, then ramp at 100C/min. to 280°C, isothermal for 12 min. Column used was HP5. The injector temperature was 200°C, and the detector temperature was 280 °C. Helium was used as carrier gas at 1 mL/min. Mass spectral scan range was set at 10 - 2000 amu. Mass resolution 6000 amu. A gas chromatograph coupled with a mass spectrometer (GC-HRMS) is a combined analyzer that has the ability to analyze compounds qualitatively and quantitatively.

The components in the extract were identified based on the mass spectra of the latest library. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library, Sophisticated Analytical Instrument Facility (SAIF) IIT Bombay.

Result and discussion

Percentage of yield

The dried powdered leaves (25g) of *P. dasycaulon* were serially extracted with Petroleum ether, chloroform, acetone, and methanol and aqueous (non- polar polar) for 7 hours, and the percentage yield was 3.036, 1.552, 3.664, 8.804, and 7.93 respectively. The differences in the extraction of yield depend on the nature of the solvents and the chemical nature of the sample. The high yield was observed in methanolic extract followed by an aqueous extract of leaves. So these methanolic extracts were selected for further GC/MS analysis. The chemical constituents present in polar methanolic extract of *P. dasycaulon* leaves was investigated using Gas Chromatography High Resolution Mass spectroscopy (GC-HRMS) (Table 1). GC/MS works on the separation of the single compound by GC according to their Retention time (rt), and the same compounds were further analyzed at a molecular level by MS detector [12]. The GC-HRMS chromatogram observed 20 peaks of the compounds as shown in chromatogram (Figure 2) and the corresponding mass fragments of each identified phytoconstituents (Figure 3 and Figure 4) as follows; 2 (3H)-Furanone dihydro-4-hydroxy, 2-Furanmethanol, 5-ethenyltetrahydro-α, α, 5-trimethyl-,cis-,DAlanine, N-propargyloxycarbonyl-isohexyl ester, 1,3,5-dihydroxy- 6-methyl-2-3-dihydro-4-pyran-4-one, Furandiol, tetrahydro-,cis, Benzoic acid, 2 butoxy, methyl ester, 2-Furancarboxaldehyde, 5 (hydroxyl, 4-hydroxy 3 methyl acetophenone, 1-(3,6,6-trimethyl-1,6,7,7 a-terahydrocyclopenta-(c)-pyran-1-yl) ethanone, Sucrose, Alpha-1-rhamnopyranose, 3,7,11,15-tetramethyl –α-hexadecen-1 ol, (6z)-6-pentadecen-1-ol, n- hexadecanoic acid, Phytol, 7,10,13- hexadecatrienal, Octadecanoic acid, E-10,13,13, trimethyl-11-tetradecen-1-ol acetate, 1,3-hydroxyspirotene-en-11-one and Octadecane, 3 ethyl-5-(2-ethyl butyl). The mass fragments along the structure of each identified compound were compared with the NIST database (Figure 5, Figure 6 and Figure 7).

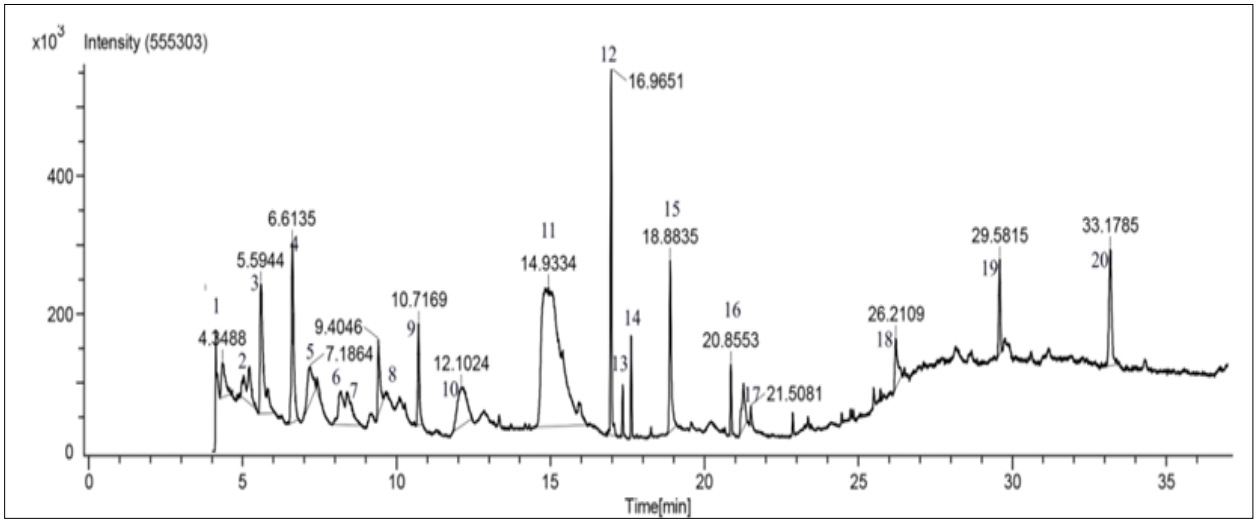

Fig 2: GC-HRMS Chromatogram of leaves methanolic extract of *Pittosporum dasycaulon*

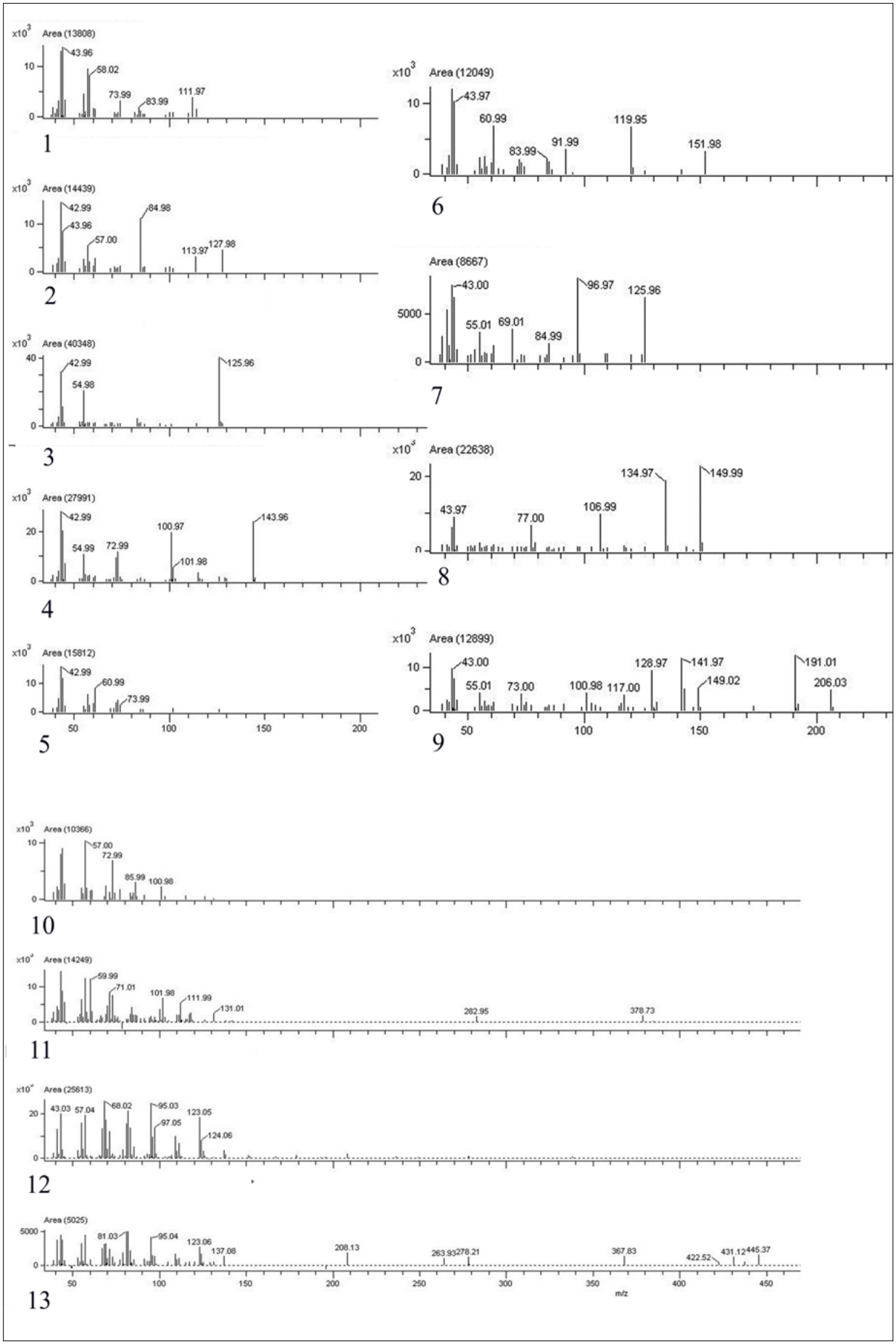

Fig 3: MS fragment of identified compounds from the crude methanolic extract of *P. dasycaulon* leaves.

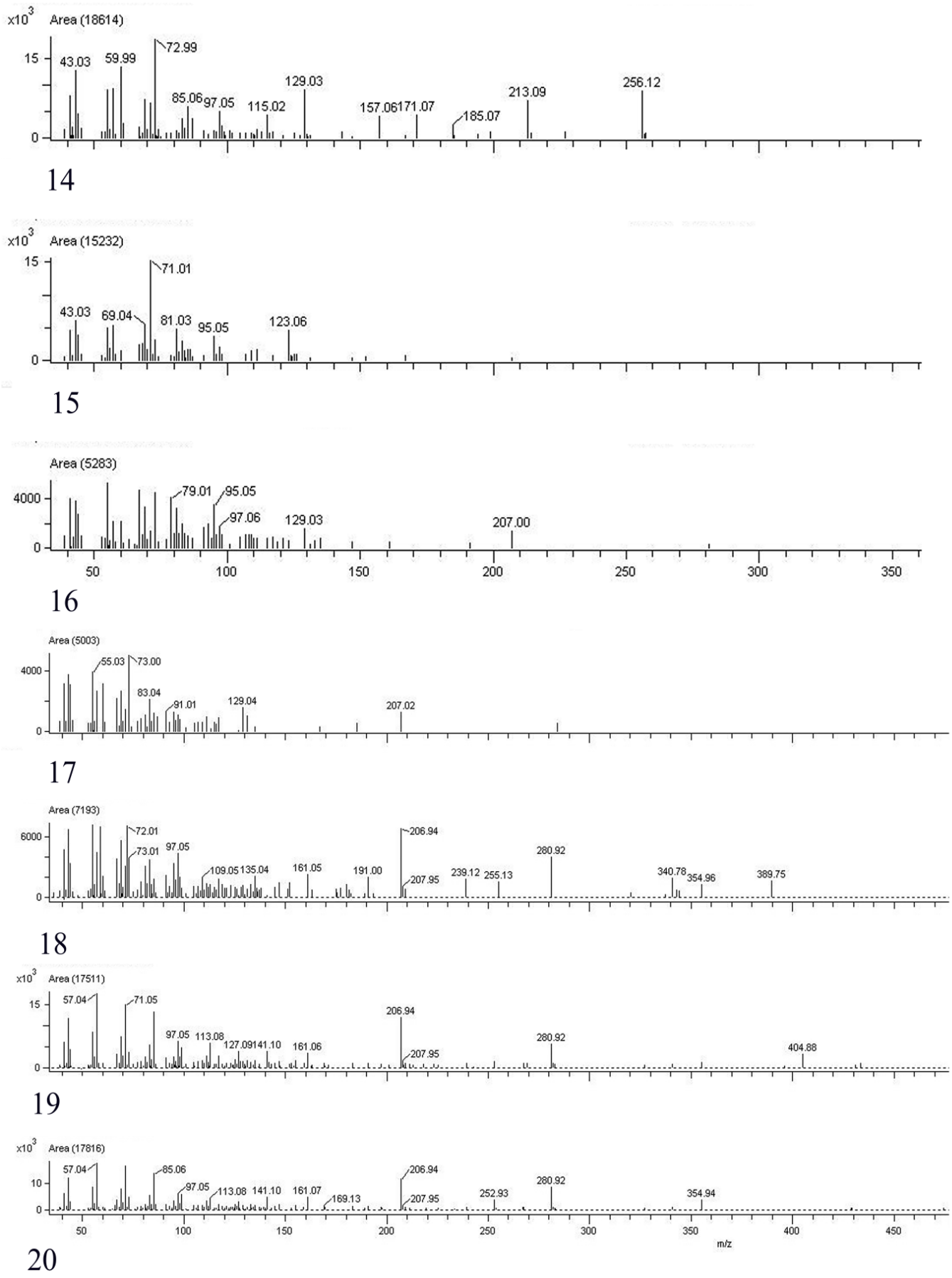

Fig 4: MS fragment of identified compounds from the crude methanolic extract of *P. dasycaulon* leaves.

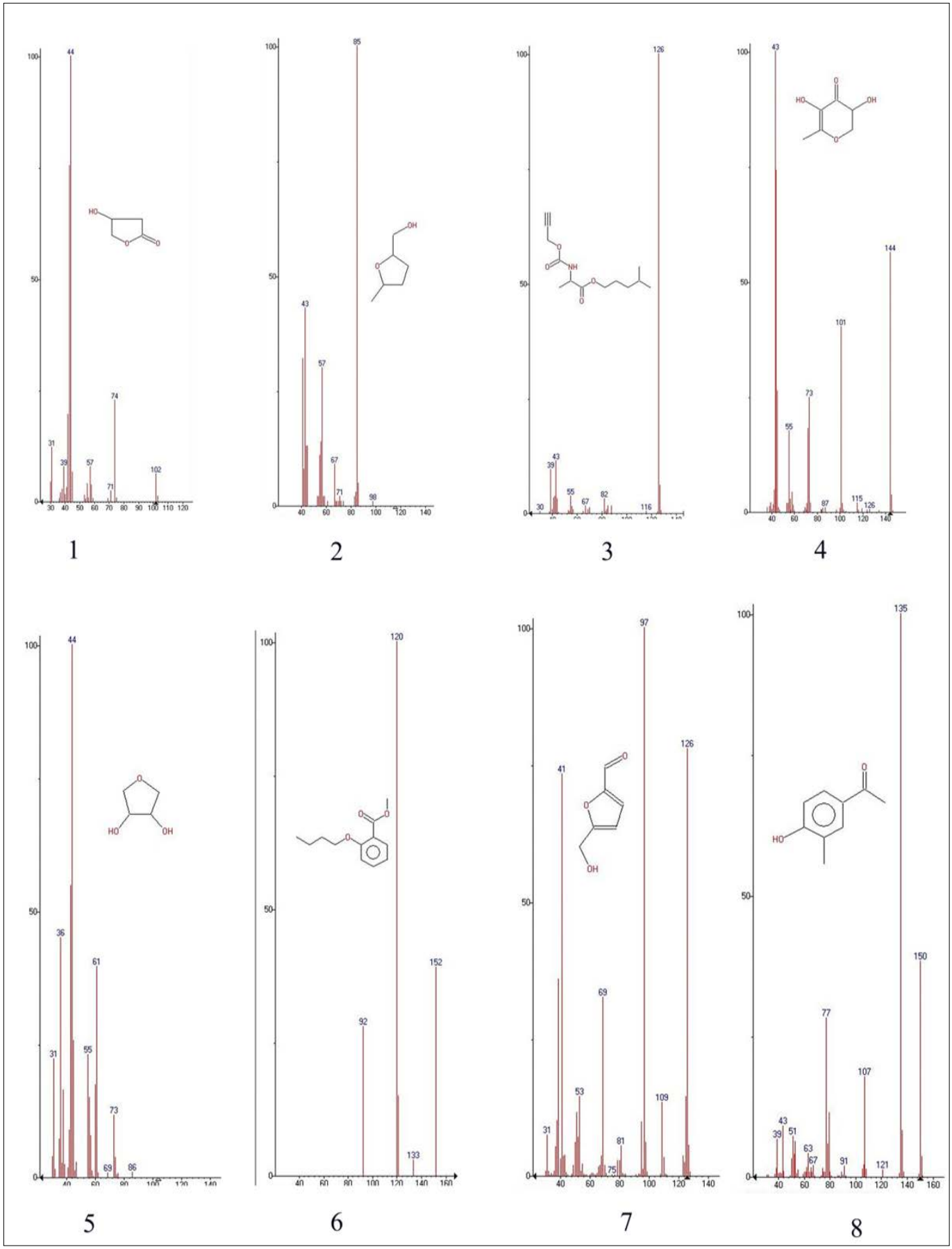

Fig 5: Depicted the standard (NIST database) Mass fragments (MF) along with structure of identified compounds. (Note: red line represents major peaks of compounds).

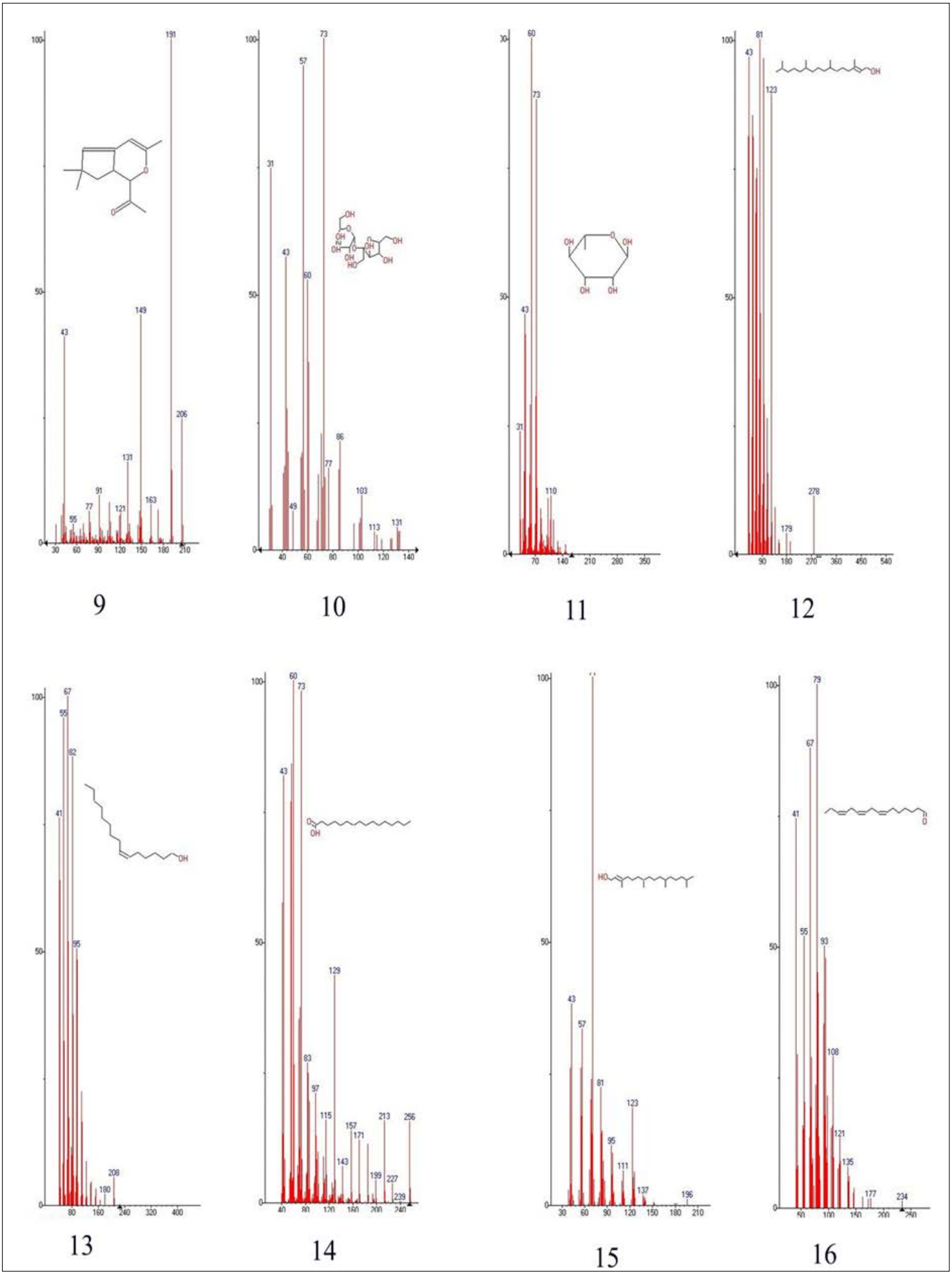

Fig 6: Depicted the standard (NIST database) Mass fragments (MF) along with structure of identified compounds. (Note: red line represents major peaks of compounds).

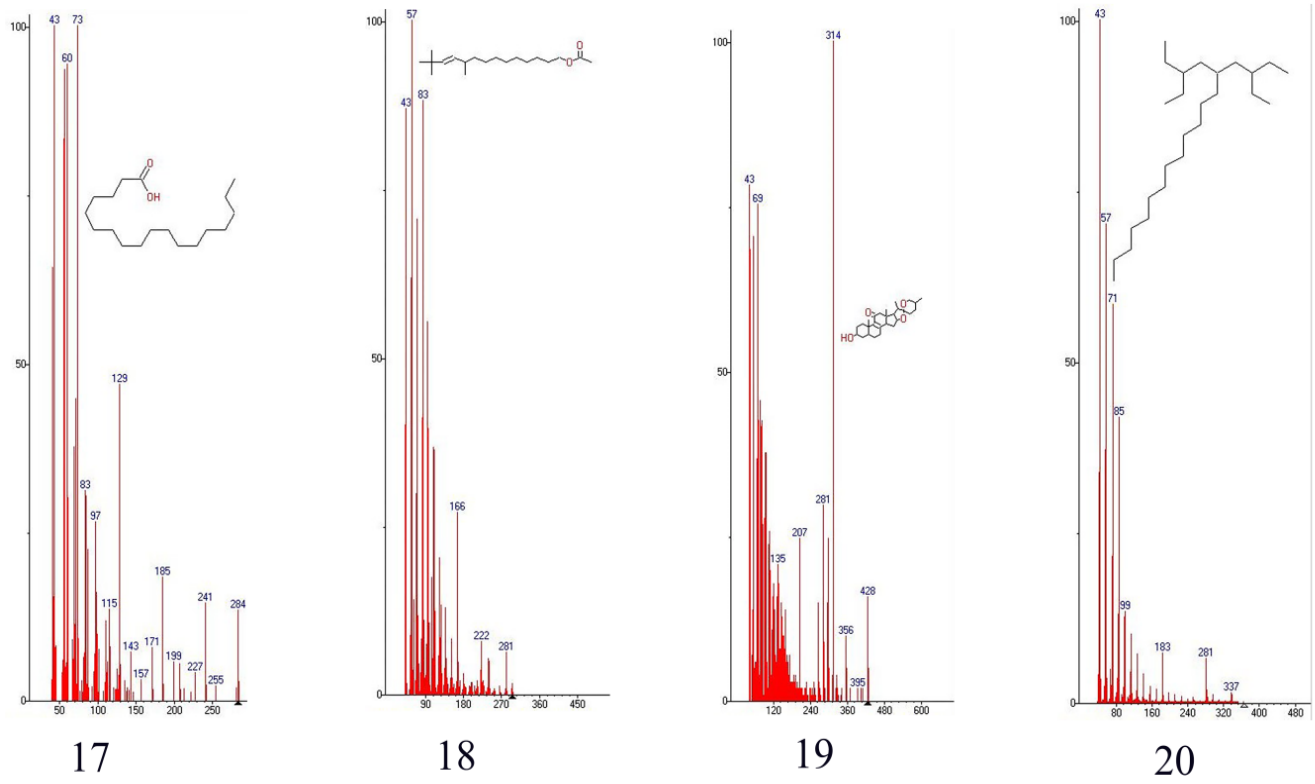

Fig 7: Depicted the standard (NIST database) Mass fragments (MF) along with the structure of identified compounds. (Note: red line represents major peaks of compounds).

Pharmacological importance of identified compounds

Rich sources of biologically potent chemicals were obtained from medicinal plants that are mostly free from adverse side effects; plant extracts may have excellent pharmacological properties [13].

Table 1: Compound identified through HR-GCMS analysis of *Pittosporum dasycaulon*.

Sl. No	Name of the compound	MW	MF	RT	Area (%)
1	2 (3H)-Furanone dihydro-4-hydroxy	102	$C_4H_{12}O_2$	4.34	2.486
2	2-Furanmethanol,5-ethenyltetrahydro-α,α,5-trimethyl-,cis-,	170	$C_6H_{12}O_2$	5.21	2.600
3	D Alanine, N-propargyloxycarbonyl-isohexyl ester	209	$C13H_{21}NO_4$	5.58	7.265
4	1,3,5-dihydroxy- 6-methyl-2-3—dihydro-4-pyran-4-one	144	$C_6H_8O_4$	6.61	5.04
5	Furandiol, tetrahydro-,cis	104	$C_4H_8O_3$	7.17	2.847
6	Benzoic acid, 2 butoxy, methyl ester	208	$C_{12}H_{16}O_3$	7.42	2.169
7	2-Furancarboxaldehyde, 5 (hydroxy	126	$C_6H_6O_3$	8.18	1.560
8	4-hydroxy 3 methyl acetophenone	150	$C_9H_{10}O_2$	9.40	4.076
9	1-(3,6,6-trimethyl-1,6,7,7 a-terahydrocyclopenta (c) pyran-1-yl) ethanone	206	$C_{13}H_{18}O_2$	10.71	2.322
10	Sucrose	342	$C_{12}H_{22}O_{11}$	12.08	1.866
11	Alpha-1-rhamnopyranose	164	$C_6H_{12}O$	14.93	2.565
12	3,7,11,15-tetramethyl –α-hexadecen-1 ol	296	$C_{20}H_{40}O$	16.96	4.612
13	(6z)-6-pentadecen-1-ol	226	$C_{15}H_{30}O$	17.34	0.904
14	n- hexadecanoic acid	256	$C_{16}H_{32}O_2$	18.88	3.352
15	Phytol	296	$C_{20}H_{40}O$	20.85	2.743
16	7,10,13- hexadecatrienal	234	$C_{16}H_{28}O$	21.26	0.951
17	Octadecanoic acid	284	$C_{18}H_{36}O_2$	21.50	0.900
18	E-10,13,13, trimethyl-11-tetradecen-1-ol acetate	296	$C_{19}H_{36}O_2$	26.20	1.295
19	1,3-hydroxyspirotene-en-11-one	428	$C_{27}H_{40}O_4$	29.58	3.153
20	Octadecane, 3 ethyl-5-(2-ethyl butyl)	366	$C_{26}H_{54}$	33.17	3.208

The previous reports suggest that 2 (3H)-Furanone dihydro-4-hydroxy was prominent in the extract of *Crocus sativus* and shows antioxidant, antifungal, and anticancer activities [14]. The organic alcohol 2-Furanmethanol, 5-ethenyltetrahydro-α, α, 5-trimethyl-, cis-, was reported for its antiviral and antioxidant activities which were identified from the methanolic extract of *Artemisia annua* [15]. The bark extract of *Juglans regia* showed prominent antimicrobial activity, GC/MS analysis of bark extract reported the presence of Furandiol, tetrahydro-, cis [16]. Lima *et al*, (2018) reported that the phenolic derivative, benzoic acid, 2 butoxy, methyl ester shows strong inhibition against fungus *Candida albicus* [17]. The anticancer activity of benzoic acid derivatives was recently investigated *in vitro* by Shibata *et al*. (2000) [18] and Rahuman *et al*. (2020) [19], through insilico study. Hexadecanoic acid and octadecanoic

acid are the main constituents of volatile oils which are found among the plants that strongly inhibit inflammations found in human endothelial cells [20-21]. Phytol is a diterpenoid alcohol that is also reported for its good antiinflammatory potential [22]. The anticancer and antidiuretic activities of phytol were also reported by Vats and Gupta, (2017) [23]. Anti-inflammatory, antimicrobial and antioxidant activities were found due to be the presence of compound 1, 3-hydroxyspirotene-en-11-one [15]. The recent study on Octadecane, 3 ethyl-5-(2-ethyl butyl) shown antinemacidal activity by [24]. Based on the available literature, most of the identified compounds from *P. dasycaulon* found to be antinflammatory followed by anticancer and antimicrobial activities. However, antiinflammatory potential of the genus *Pittosporum* was studied by several authors [3, 8, 9, 25, 26]. The present HR-GCMS analysis were also supported the present phytochemical investigations. The rest of phytochemicals that were identified in the present study, including D Alanine, N-propargyloxycarbonyl-isohexyl ester, 1,3,5-dihydroxy- 6-methyl-2-3-dihydro-4-pyran-4-one, Furandiol, tetrahydro-,cis, 2-Furancarboxaldehyde, 5 (hydroxyl, 4-hydroxy 3 methyl acetophenone, 1-(3,6,6-trimethyl-1,6,7,7 a-terahydrocyclopenta (c) pyran-1-yl) ethanone, 3,7,11,15-tetramethyl –α-hexadecen-1 ol, and (6z)-6-pentadecen-1-ol have no sufficient attention towards the pharmacological value. To the best of our knowledge, this is the first report on the high-resolution GC-MS method in the plant. In addition, we were able to identify 20 polar compounds based on their retention times, retention indices, and fragmentation patterns. So further *in vitro* and *in vivo* studies are needed to establish the pharmacological value of the methanolic extract of *P. dasycaulon*.

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References

1. Singh RK. Lectotypification of three Indian endemic taxa of Pittosporum (Pittosporaceae). Turkish Journal of Botany,2016:40(5):557-60.
2. Joshi B, Panwar GS, Ambrish K. Seed germination and seed storage behavior of Pittosporum eriocarpum Banks. ex Gaertn.: An endemic species of North-West Himalaya. Indian Journal of Forestry,2019:42(3):203.
3. Mani B, Dennis T. Evaluation of the antioxidant potential of Pittosporum dasycaulon Miq. Stem bark. Food Science and Biotechnology,2014:23(2):539-45.
4. Mani B, Thomas TD. Evaluation of Antibacterial Potential of Stem Bark of Ethnomedicinal Tree Pittosporum dasycaulon Miq. International Journal of Plant Science and Ecology,2015:1(3):113-8.
5. Abdullah Al-Dhabi N, Valan Arasu M, Rejiniemon TS. In vitro antibacterial, antifungal, antibiofilm, antioxidant, and anticancer properties of isosteviol isolated from endangered medicinal plant Pittosporum tetraspermum. Evidence-Based Complementary and Alternative Medicine, 2015, 26.
6. Rosakutty PJ, Roslin AS. Isolation and characterization of an antimicrobial compound from the traditional medicinal plant Pittosporum tetraspermum Wight & Arn. Int J Med Aromat Plants,2012:2(1):141-150.
7. Bäcker C, Jenett-Siems K, Siems K, Niedermeyer TH, Wurster M, Bodtke A *et al*. Taraxastane-type triterpene saponins isolated from Pittosporum angustifolium Lodd. *Zeitschrift für Naturforschung B*,2015:70(6):403-408.
8. Mendes SA, Mansoor TA, Rodrigues A, Armas JB, Ferreira MJ. Anti-inflammatory guaiane-type sesquiterpenes from the fruits of Pittosporum undulatum. Phytochemistry,2013:1(95):308-14.
9. Madikizela B, McGaw LJ. In vitro cytotoxicity, antioxidant and anti-inflammatory activities of Pittosporum viridiflorum Sims and Hypoxis colchicifolia Baker used traditionally against cancer in Eastern Cape, South Africa. South African Journal of Botany,2019:1:126:250-5.
10. Pavithra K, Vadivukkarasi S. Evaluation of free radical scavenging activity of various extracts of leaves from Kedrostis foetidissima (Jacq.) Cogn. *Food Science and Human Wellness*,2015:4(1):42-46.
11. Deshmukh MM, Ambad CS, Kadam MR, Kashid NG. Evaluations of 2-hydroxy 4-methoxy benzaldehyde from petroleum ether extract of hemidesmus indicus (l) by using thin layer chromatography and gc-hrms techniques. International Research Journal of Modernization in Engineering Technology and Science,2020:2(11):769-778.
12. Painuli SA, Rai NI, Kumar NA. Gas chromatography and mass spectrometry analysis of a methanolic extract of leaves of Rhododendron arboreum. GAS,2016:9(1):101-4.
13. Melo MM, Habermehl GG, Oliveira NJF, Nascimento EF, Santos MMB, Lúcia M. Treatment of Bothrops alternatus envenomation by Curcuma longa and Calendula officinalis extracts and ar-turmerone. Arquivo Brasileiro de Medicina Veterinária e Zootecnia,2005:57:7-17.
14. Zheng CJ, Li L, Ma WH, Han T, Qin LP. Chemical constituents and bioactivities of the liposoluble fraction from different medicinal parts of Crocus sativus. Pharmaceutical biology,2011:49(7):756-63.
15. Hameed IH, Altameme HJ, Idan SA. Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. Research journal of pharmaceutical biological and chemical sciences,2016:7(2):1843-68.
16. Ara I, Shinwari MM, Rashed SA, Bakir MA. Evaluation of antimicrobial properties of two different extracts of Juglans regia tree bark and search for their compounds using gas chromatography-mass spectrum. International Journal of Biology,2013:5(2):92.
17. Lima TC, Ferreira AR, Silva DF, Lima EO, De Sousa DP. Antifungal activity of cinnamic acid and benzoic acid esters against Candida albicans strains. Natural product research,2018:32(5):572-5.
18. Shibata J, Murakami K, Wierzba K, Aoyagi Y, Hashimoto A, Sano M *et al*. Anticancer effect of 4-[3, 5-bis (trimethylsilyl) benzamido] benzoic acid (TAC-101) against A549 non-small cell lung cancer cell line is related to its anti-invasive activity. Anticancer research,2000:20(5A):3169-76.
19. Rahuman MH, Muthu S, Raajaraman BR, Raja M. Quantum computational, spectroscopic and molecular docking investigations on 4-Acetylamino-benzoic acid

methyl ester: A prospective anticancer drug, Chemical Data Collections 26, 100352
20. de Souza CO, Valenzuela CA, Baker EJ, Miles EA, Rosa Neto JC, Calder PC. Palmitoleic acid has stronger anti-inflammatory potential in human endothelial cells compared to oleic and palmitic acids. Molecular nutrition & food research,2018:62(20):1800322.
21. Othman AR, Abdullah N, Ahmad S, Ismail IS, Zakaria MP. Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from Jatropha curcas L. plant root. BMC complementary and alternative medicine,2015:15(1):1-0.
22. Olofsson P, Hultqvist M, Hellgren LI, Holmdahl R. Phytol: A chlorophyll component with anti-inflammatory and metabolic properties. InRecent Advances in Redox Active Plant and Microbial Products Springer, Dordrecht, 2014, 345-359.
23. Vats S, Gupta T. Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of Moringa oleifera Lam. from Rajasthan, India. Physiology and molecular biology of plants,2017:23(1):239-48.
24. Akshaya SB, Krishnamoorthy AS, Nakkeeran S, Poornima K, Siva kumar U. "Inhibitory potential of ethyl acetate extract from mushrooms against root-knot nematode (Meloidogyne incognita)." J Entomol Zool Stud,2021:9(1):528-534
25. Rosakutty PJ, Roslin AS, Ignacimuthu S. Anti-inflammatory and acute toxicity effects of Pittosporum tetraspermum Wight and Arn. on rats. Journal of Phytology,2010:2(6):14-20.
26. Wandji BA, Bomba FD, Nkeng-Efouet PA, Piegang BN, Kamanyi A, Nguelefack TB. Anti-hyperalgesic activity of the aqueous and methanol extracts of the leaves of Pittosporum mannii Hook on CFA-induced persistent inflammatory pain. Inflammopharmacology,2018:26(1):197-205.