ORIGINAL ARTICLE

ScienZTech

INTERNATIONAL JOURNAL OF NOVEL TRENDS IN PHARMACEUTICAL SCIENCES

Published by ScienzTech Publication Journal Home Page: www.scienztech.org/ijntps

Phytochemical screening and acute oral toxicity study of *Coldenia procumbens* and *Mukia maderaspatana* whole-plant extracts

Ramachandran K*[1], Venketnarayanan R[2], Rajesh Yadav[3], Suresh R[2], Sumitra Devkota[4]

[1]Department of Pharmacy, College of Medical Sciences, Bharatpur-44200, Chitwan District, Nepal
[2]Department of Pharmacology, R. V. S. College of Pharmaceuticals Sciences, Coimbatore-641402, Tamilnadu, India
[3]Department of Anesthesiology, College of Medical Sciences and Teaching Hospital, Bhartapur-44200, Chitwan, Nepal
[4]Nepalese Army Institute of Health Sciences, Sanobharyang-44600, Kathmandu, Nepal

Article History:

Received on: 09 May 2019
Revised on: 15 Jun 2019
Accepted on: 26 Jun 2019
Published on: 06 Jul 2019

Volume: 9 Issue: 2

Keywords:

Coldenia procumbens,
Mukia maderaspatana,
Phytochemistry,
NMR,
IR

ABSTRACT

Usage of drugs to stop the growth of cancer cells both by killing the cancer cells or by stopping them from dividing is known as chemotherapy. Even though so many anticancer drugs are available in the market, there are many side effects of these drugs and therefore, the patient requires secondary palliative care. The way the chemotherapy is given depends on the type and stage of the cancer being treated. It plays an essential role in the discovery of lead compound for the development of conventional drugs. Plant herbs have non-toxic side effects. The plant *Coldenia procumbenslinn* and *Mukia maderaspatana* (L) M. Roem possess chemical compounds that show antioxidant and anticancer activity.,Mukia maderaspatanan (L) M. Roem, Coldenia procumbens linn before its clinical usage, through the toxicological profile, was determined on the crude extracts as well as on isolated compounds to confirm the safety of the drug.

*Corresponding Author

Name: Ramachandran K
Phone: +9779808878838
Email: rcrama911@gmail.com

eISSN: 2277-2782

DOI: https://doi.org/10.26452/ijntps.v9i2.1194

Production and Hosted by

ScienzTech.org

INTRODUCTION

Usage of drugs to stop the growth of cancer cells both by killing the cancer cells or by stopping them from dividing is known as chemotherapy. Oral, Intramuscular and intravenous drugs(systemic chemotherapy) enter bloodstream an reach the cancer cells of the body. When chemotherapy is injected to CSF directly, or into an organ, or body cavity such as the abdomen, the drugs will affect cancer cells in those particular areas only. It is called Regional chemotherapy [1] .

If cancer spreads to the liver, chemoembolization of the hepatic artery is beneficial for treating cancer. The process involves blockage of hepatic artery and injection of anticancer drugs between the blockage and liver. The drug reaches the liver through the arteries of livers and only minute amounts of the drug enter into other parts of the body. The blockage of the hepatic artery may be temporary or permanent and the liver receives blood via the hepatic portal vein.

Even though so many anticancer drugs are available in the market, there are many side effects of these drugs and therefore, the patient requires secondary palliative care. The way the chemotherapy is given depends on the type and stage of the cancer being treated. It plays an essential role in the discovery of lead compound for the development of conventional drugs. About 60% of currently used anti

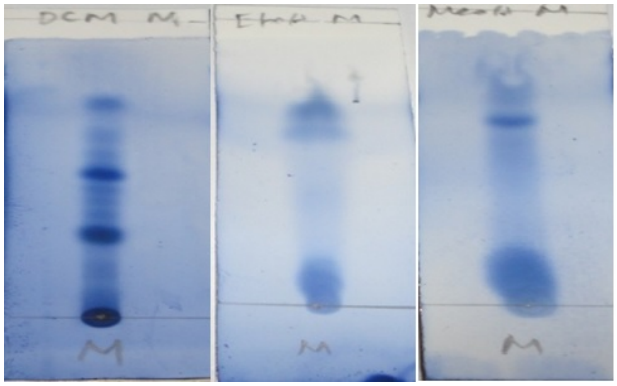

Figure 1: TLC of *Mukia madersapatana* on DMP, CMP, MMP

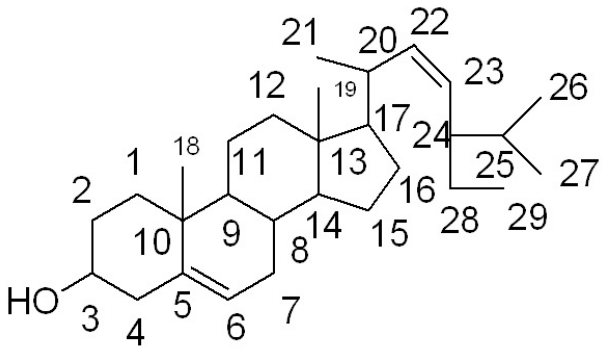

Figure 2: Stigmasterol ring structure

Figure 3: Fatty ester structure

cancer agents are derived from a natural source (i.e. plants).

Plant herbs have non-toxic side effects. The primary objective of the study is to develop a drug from the medicinal plant to treat colon cancer with non-toxic side effects and to elucidate their structures using advanced techniques. In the present study, *Coldenia procumbens* and *Melothoria maderaspatana* were studied for their phytoconstituents and structural elucidation of the compounds. Their acute toxicity was also evaluated.

Coldenia procumbens is a herb lying flat on the ground, common on dry rice grounds. The stem reaches to 10-50 cm long and it is shaggy with white hairs. The stem produces numerous branches. It is most commonly seen in South India especially in tropical and subtropical zones of South India. Leaves are alternate, short, sessile, crisped and rounded at the apex, and the basal leave are rosette with veins of 4-6 pairs on each side. The leaves are most often used as poultice to mature abscess and also applied to rheumatoid arthritis patients for reducing swellings. Oil is most commonly used as a liniment to knees and joints which are swollen.

Melothoria maderaspatana is an annual climber with hairy shoots. Leaves simple, alternative, deltoid or sometimes ovate, 3-5 lobed, with scrubby hairs all around. Flowers small yellow, unisexual, fruits globose, greenish with streaks when unripe and bright red when ripe, with short, numerous compressed ovoid seeds. The parts of the plant are used as vermifuge and febrifuge. It is also used as animal fodder. Leafy shoots of the plant are used as laxatives.

MATERIALS AND METHODS

Collection and identification of plant material

Coldenia procumbens, the whole plant and *Mukia maderaspatana* were collected in Tirunelveli district of Tamilnadu in India. The plant was confirmed by botanical identity and also authenticated by a Taxonomist Dr. V. Chelladurai (Research officer, Botany, C. C. R. A. S) Government of India.

Preparation of various extracts of the Plant *Coldenia procumbens* and *Mukia maderaspatana*

150 grams of plant powder was packed in Soxhlet apparatus and it is refluxed with Dichloroethane until it gets a clear solution then the marc was pressed. This percolation of two plant extraction process was continued with other solvents Ethanol, Methanol and Water. The pooled the two extracts of different solvents were concentrated to get dry the two extracts by evaporation. Then these the two extracts were designated as DCP, ECP, MCP, ACP of Coldenia procumbens and DMP, EMP, CMP, AMP of Mukia maderaspatana and utilized in the present study

Pharmacognostic Study

Morphology

The physical and external structure of both the plants includes the shape, size, colour, texture and order of the crude drug or in the powder form were studied. This study helps to identify the crude drug.

Preliminary phytochemical studies

A known quantity of dried extract of *Coldenia procumbens* and *Mukia maderaspatana* extracts were dissolved in the respective solvent and used in the following qualitative tests [1] .

Determination of total phenols

The total phenol content of the extracts was determined by using the Folin-ciocalteu assay method. An

aliquot (1ml) of the two extract or std solution of gallic acid (5,10,20,40,80μg) were mixed with 0.2ml of Folin-ciocalteu reagent [2]. After 5 min, 1ml of 15% Na_2CO_3 solution and 2ml of distilled water was added to the above mixture. Reagent blank using distilled water was prepared. After incubation for 90 min at room temperature, the absorbance of the mixture was measured against prepared reagent blank at 740 nm. Samples were analyzed in triplicate. The mean of three readings was used and the total phenolic content was expressed as mg/g the two extract.

TLC Analysis

The various extracts obtained using various solvents such as Dichloromethane, Ethanol, Methanol and water were subjected to TLC Analysis to identify the active compounds present in the two plants.TLC plates were prepared using silica gel G slurry and activated at 80-120º C for 30 minutes. Spots of various the extracts were placed and kept in sample saturation chamber using different mobile phases Toluene: Ethyl acetoacetate (9.3:0.7); Chloroform: Ethyl acetoacetate (9:1); Chloroform: Ethyl acetoacetate: water (4:4:2). Ten minutes after the development period, plates dried in a hot air oven at 60º C and visualized under 254 nm [3] .

Animals

Swiss albino mice weighing 20-25gm were used for the study. Animals were fed with standard pellet and water ad libitum and maintained at temperature 24-28^{0}c temperature. 60-70% humidity and 12hr day and night cycle. Animals have fasted as described by depriving feed for 16hrs but had free access to water.

Procedure

The acute oral toxicity study was conducted using the limit test procedure according to OECD Test Guidelines 423 using female mice. Four groups at the dose levels of 5, 50, 300, 2000 mg/kg consisting of 03 animals in each group were used; animals received a single dose by intragastric intubations start with 5 mg/kg of the two plant extracts (DCP and DMP) dissolved in distilled water and observed for mortality, signs of gross toxicity or behavioural changes (excitability, convulsions, lethargy, sleep) one to four-hour post-dosing and at least once daily for 14 days for the immediate and delayed toxicity during the observation period [4] .

Isolation of compounds from Dichloromethane extract:

IR Spectral Study

IR spectra can be used for solids, liquids, or gases and for the atmospheric pollution studies IR gas analysis is a standard tool. But it is limited as it is expensive and delicate cells are needed. For liquids and solids, IR spectra can be obtained by dissolving the sample in IR transparent solvent such as CCl4 and by using simple liquid cells [5] .

Make stable to a fine paste with NUJOL^{TM} (a mixture of highly purified hydrocarbons) and 'mull' is studied directly. It exhibits a few sharp peaks which can be ignored by examining the spectrum of mull, by mixing with dry solid Kbr and grinding into a fine powder. Form a disk of the mixture by applying high pressure in a designed device and Solid spectra can be obtained.

'KBr Disk' will produce extraneous peaks which are almost spectrum free. By IR liquids can be easily analyzed.

Place Pure liquid sample (1-2 drops) between two disks of pure NaCl or Kbr and the resulting 'sandwich' was placed in the spectrometer sample holder. In this method, excellent spectra can be obtained with less expense. This is called running a **"neat"** range, which means the frequency is for the pure liquid without any solvent.

NMR Study

Samples should be dissolved in ca. 0.6-0.7 ml of a deuterated solvent and then filtered through a Pasteur pipette, equipped with a glass wool plug, that is charges into an NMR tube [6] . The purpose of filtration is to remove any undissolved sample, particulates, dust, hairs etc. from the solution, any of which may adversely affect the resolution and lineshape of your NMR spectrum. The spin magnetic moments orientate themselves in a magnetic field, although they do not all occupy the lowest possible energy state. This is a simple consequence of competition between thermal motion and the Boltzmann distribution [7] .

Mass Spectrometer Study

Multiple ions can be generated from the mass spectrometer and then separates based on the specific mass-to-charge ratio (m/z). Then it records the abundance of each ion. [8] . Electron ionization is the first step in the mass spectrometric analysis by producing the gas phase of ions. The primary production derived from the molecular ion will undergo fragmentation, and so on [9] . It results in the formation of a plot of ion abundance versus mass-to-charge ratio. The nature and structure of the precursors can be identified by the ions which are formed in mass spectroscopy [10] . In the spectrum of a pure compound, the highest value of m/z and thereby gives the molecular mass of the compound.

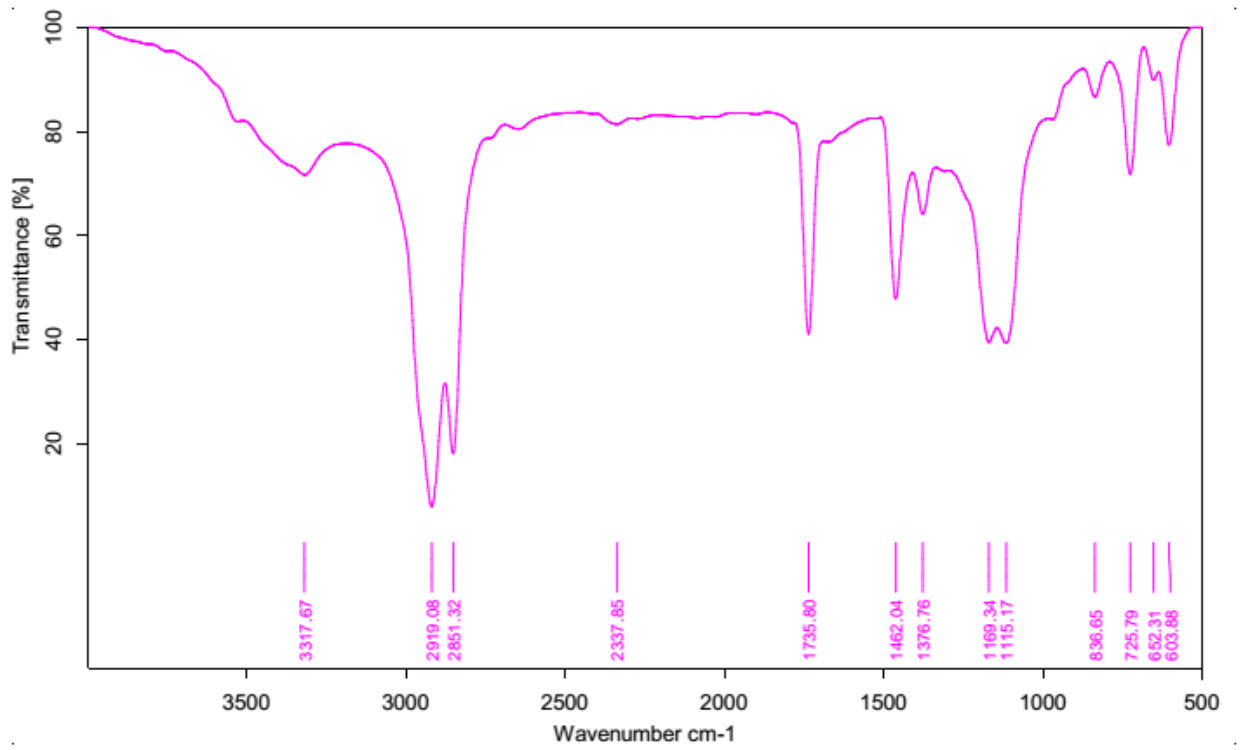

Figure 4: IR spectra of Long-chain fatty ester compound of DCP

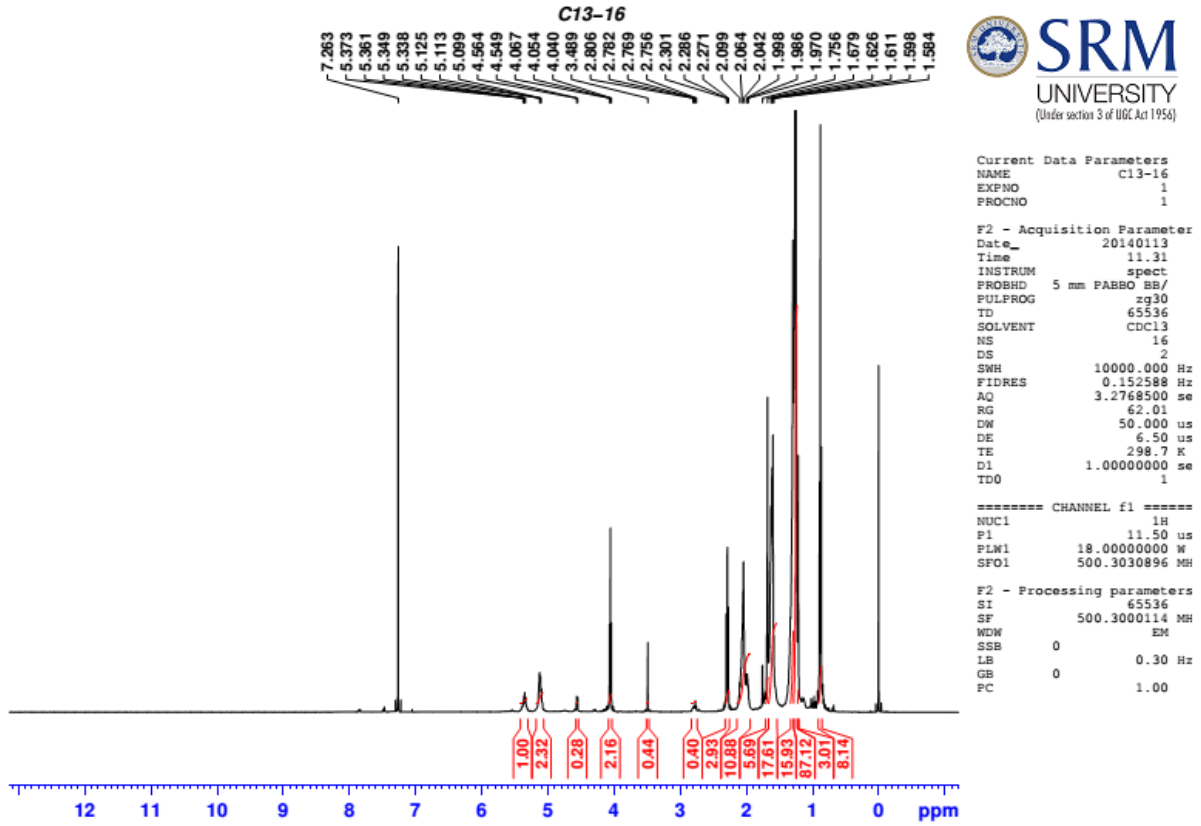

Figure 5: 500 MHZ ^{1} H NMR of Long-chain fatty ester in CDC 13 of DCP

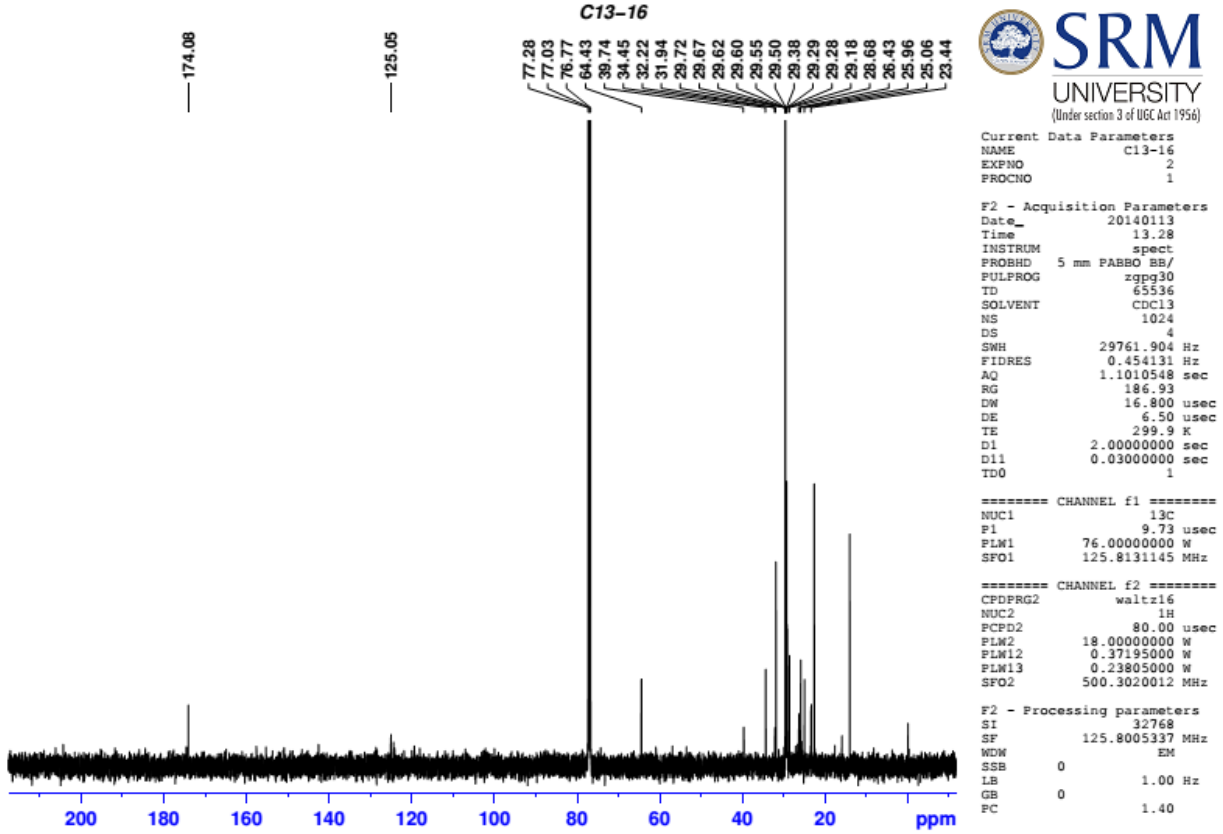

Figure 6: 125 MHZ ^{13} C NMR of Long-chain fatty ester in CDC 13 of DCP

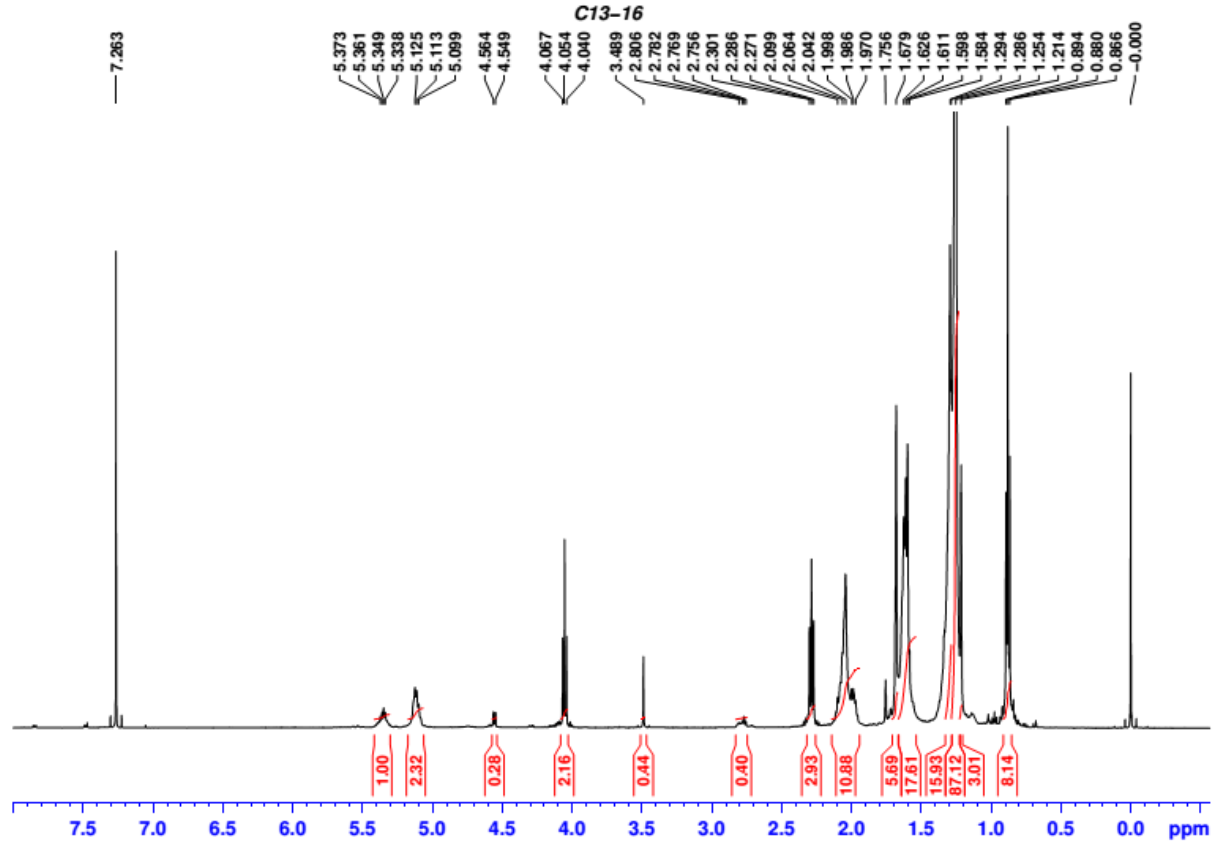

Figure 7: 125 MHZ ^{13} C NMR of Long-chain fatty ester in CDC 13 of DCP

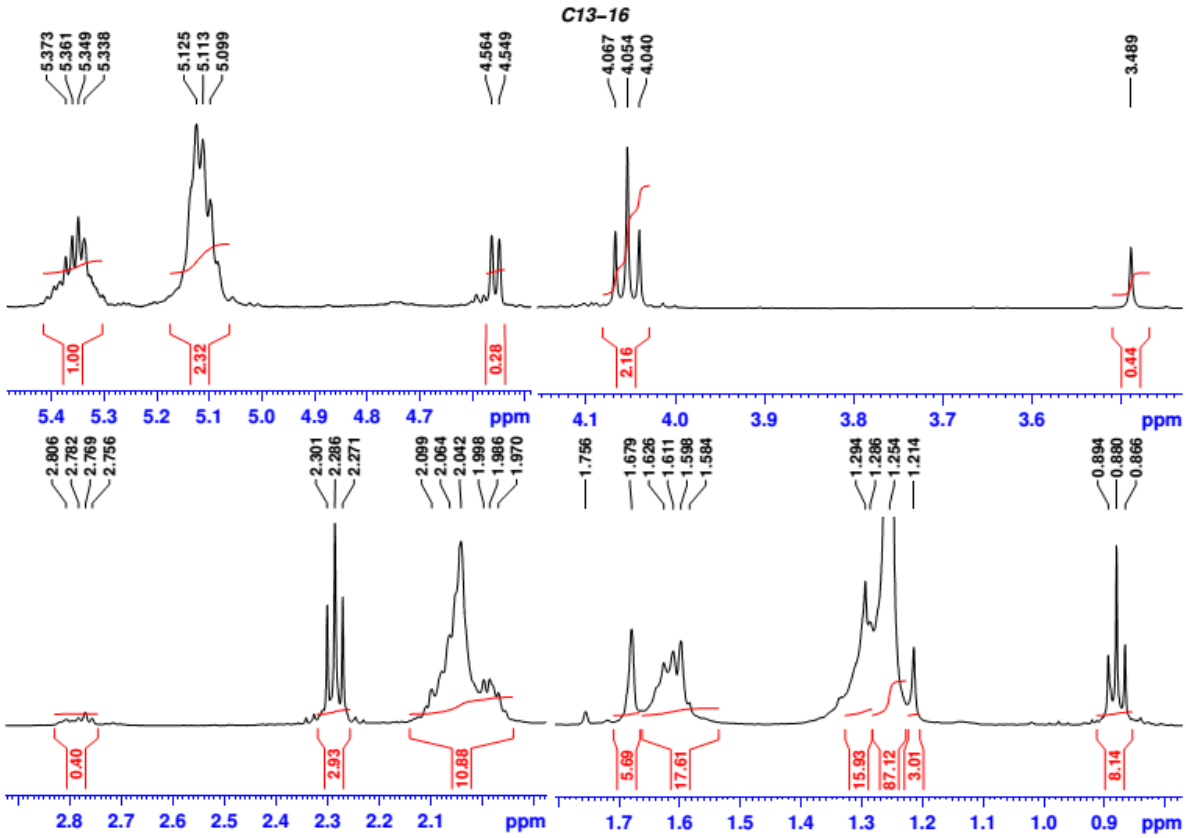

Figure 8: 125 MHZ ^{13} C NMR of Long-chain fatty ester in CDC 13 of DCP

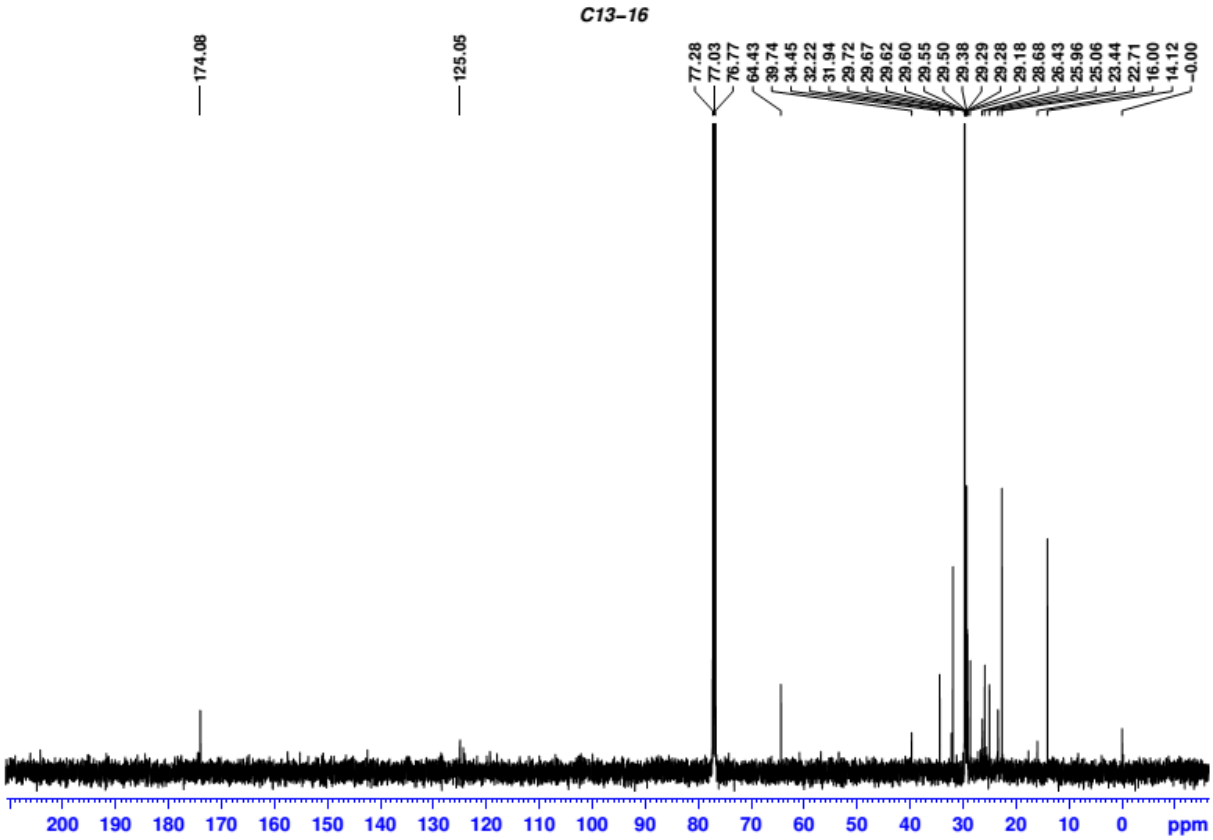

Figure 9: 125 MHZ ^{13} C NMR of Long-chain fatty ester in CDC 13 of DCP

Table 1: Preliminary phytochemical study Report

S. No	Secondary Metabolite	RESULT							
		DCP	ECP	MCP	ACP	DMP	EMP	MMP	AMP
1	Alkaloids	+	+	+	+	-	+	+	-
2	Triterpenoids	-	-	-	-	+	+	-	-
3	Carbohydrates	+	+	+	+	-	-	-	-
4	Steroids &Sterols	-	-	-	-	-	+	+	+
5	Flavanoids	+	-	-	-	+	+	-	-
6	Tannins	+	-	-	-	+	+	+	+
7	Protein &Amino acid	+	-	-	-	-	-	-	-
9	Phenol	+	+	-	-	-	-	-	-

Table 2: Rf values of Coldenia procumbens on DCP, ECP, MCP

DCM Extract	Rf value	MeOH Extract	Rf value	EtOH Extract	Rf value
1	0.31	1	0.08	1	0.09
2	0.36	2	0.20	2	0.74
3	0.56	3	0.36	3	0.87
4	0.65	4	0.47		
5	0.74	5	0.65		
6	0.80	6	0.76		
		7	0.87		

Table 3: Rf values ofMukia maderaspatana on DMP, EMP, MMP

DCM Extract	Rf value	MeOH Extract	Rf value	EtOH Extract	Rf value
1	0.07	1	0.11	1	0.12
2	0.16	2	0.65	2	0.14
3	0.20	3	0.76	3	0.45
4	0.27	4	0.80	4	0.69
5	0.34			5	0.76
6	0.41				
7	0.47				
8	0.50				
9	0.62				
10	0.69				
11	0.72				
12	0.80				

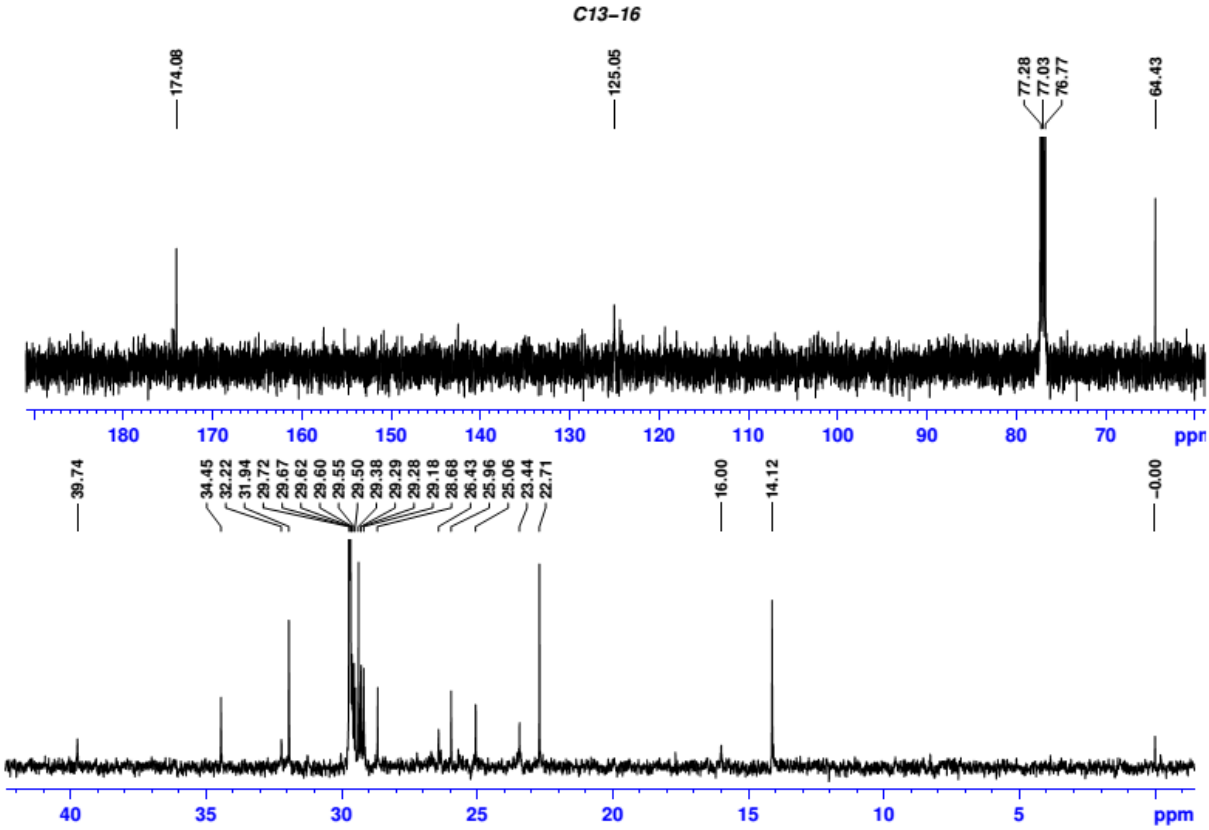

Figure 10: 125 MHZ ^{13} C NMR of Long-chain fatty ester in CDC 13 of DCP

Table 4: Screening of Acute Oral Toxicity Study Result

S. No	Response	The concentration of MCP and MMP			
		5mg/kg	50mg/kg	300mg/kg	2000mg/kg
1	Motor activity	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent
3	Touch response	Absent	Absent	Absent	Absent
4	Torch response	Normal	Normal	Normal	Normal
5	Pain response	Normal	Normal	Normal	Normal
6	Tremors	Absent	Absent	Absent	Absent
7	Convulsion	Absent	Absent	Absent	Absent
8	Righting reflux	Normal	Normal	Normal	Normal
9	Gripping strength	Normal	Normal	Normal	Normal
10	Pinna reflux	Present	Present	Present	Present
11	Corneal reflux	Present	Present	Present	present
12	Writhing	Absent	Absent	Absent	Absent
13	Pupils	Normal	Normal	Normal	Normal
14	Urination	Normal	Normal	Normal	Normal
15	Salivation	Normal	Normal	Normal	Normal
16	Skin colour	Normal	Normal	Normal	Normal
17	Lacrimation	Normal	Normal	Normal	Normal

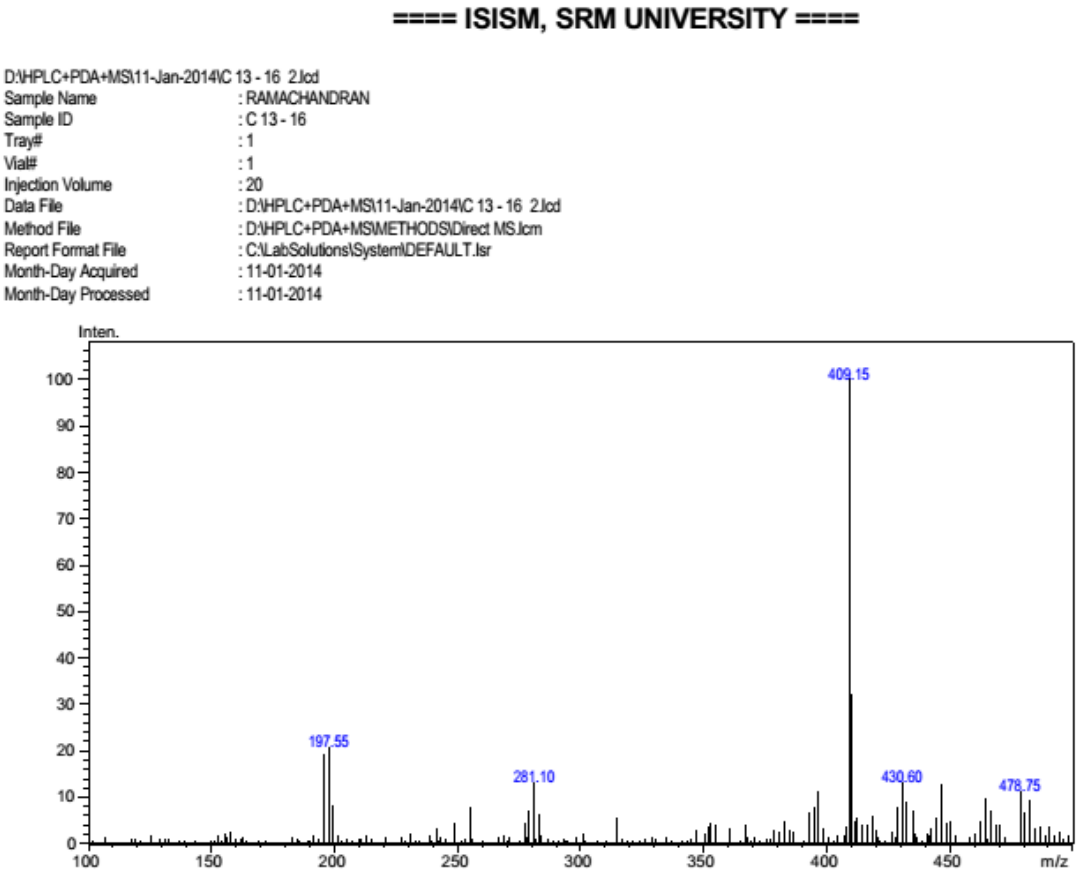

Figure 11: MASS spectra of DCP sample ID C13 - 16

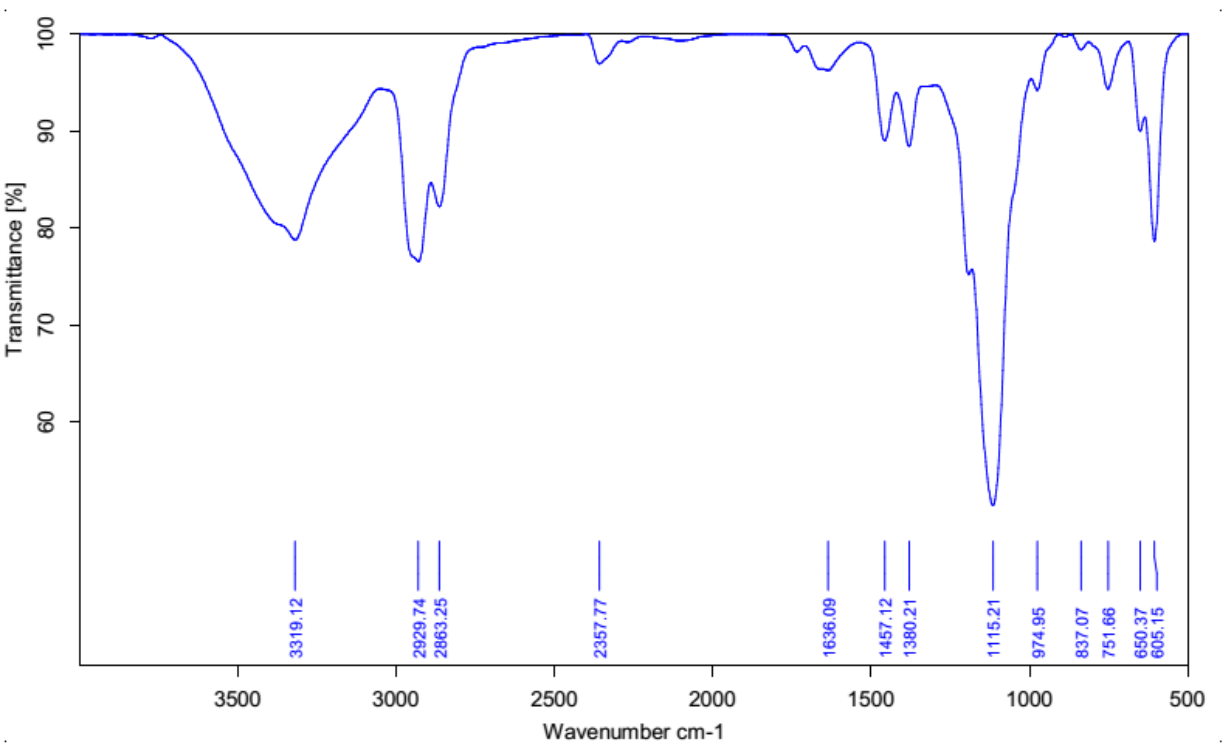

Figure 12: IR spectra of Stigmasterol compound of DMP

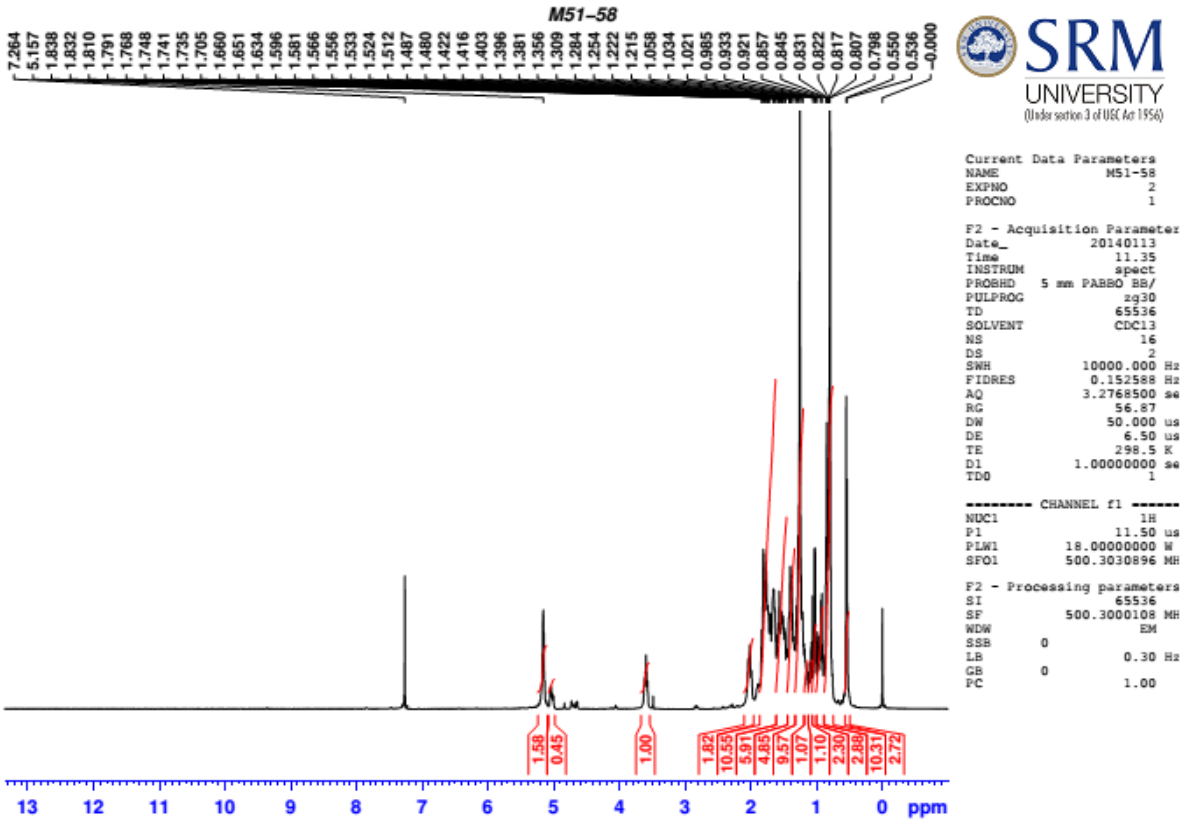

Figure 13: 500 MHZ ^{1} H NMR of Stigmasterol in CDC 13 of DMP

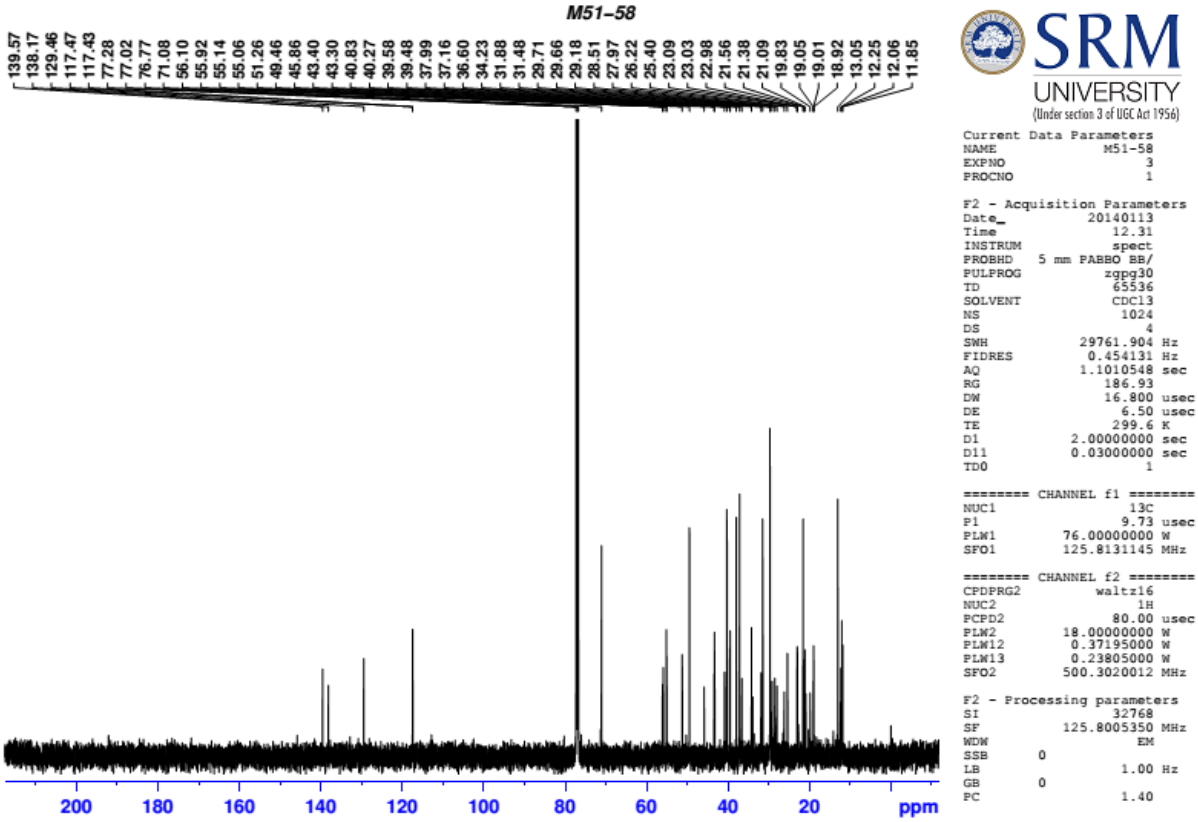

Figure 14: 125 MHZ ^{13} C NMR of Stigmasterol in CDC 13 of DMP

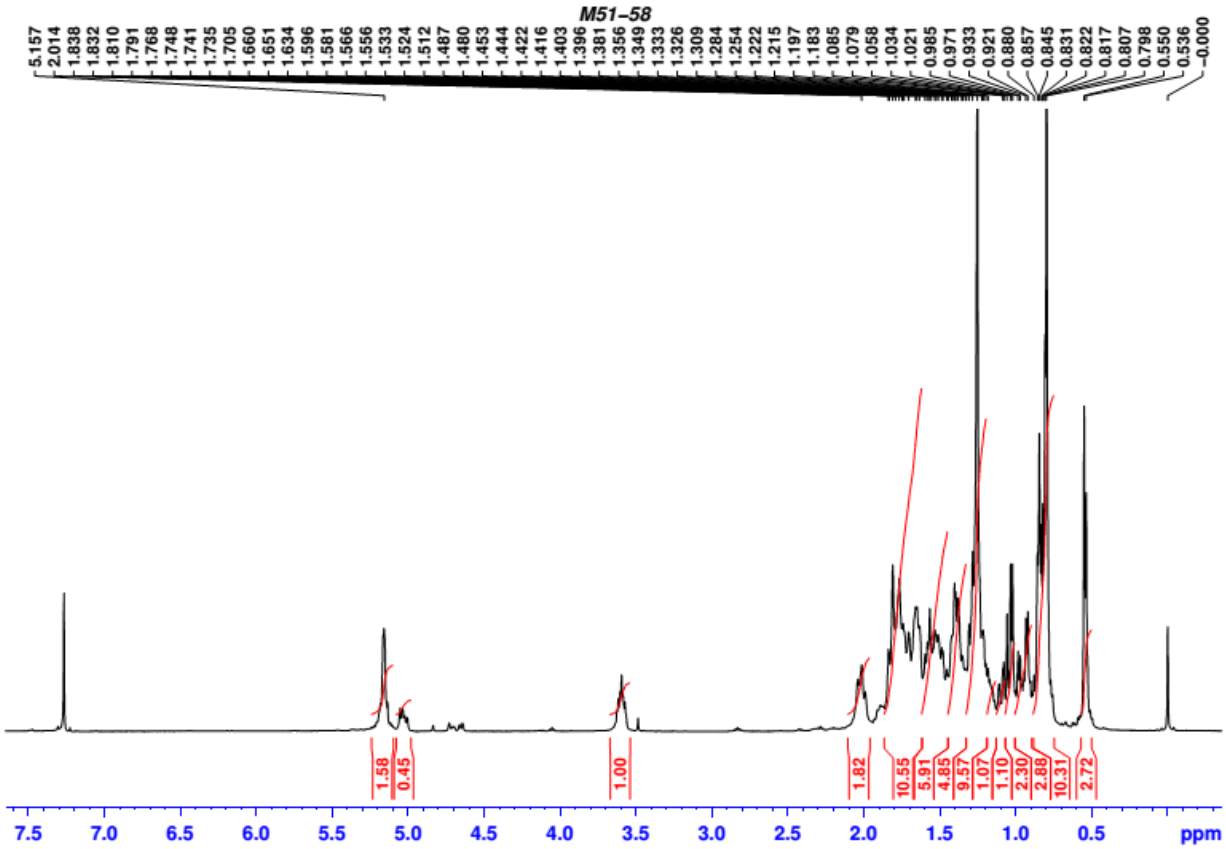

Figure 15: 125 MHZ ^{13} C NMR of Stigmasterol in CDC 13 of DMP

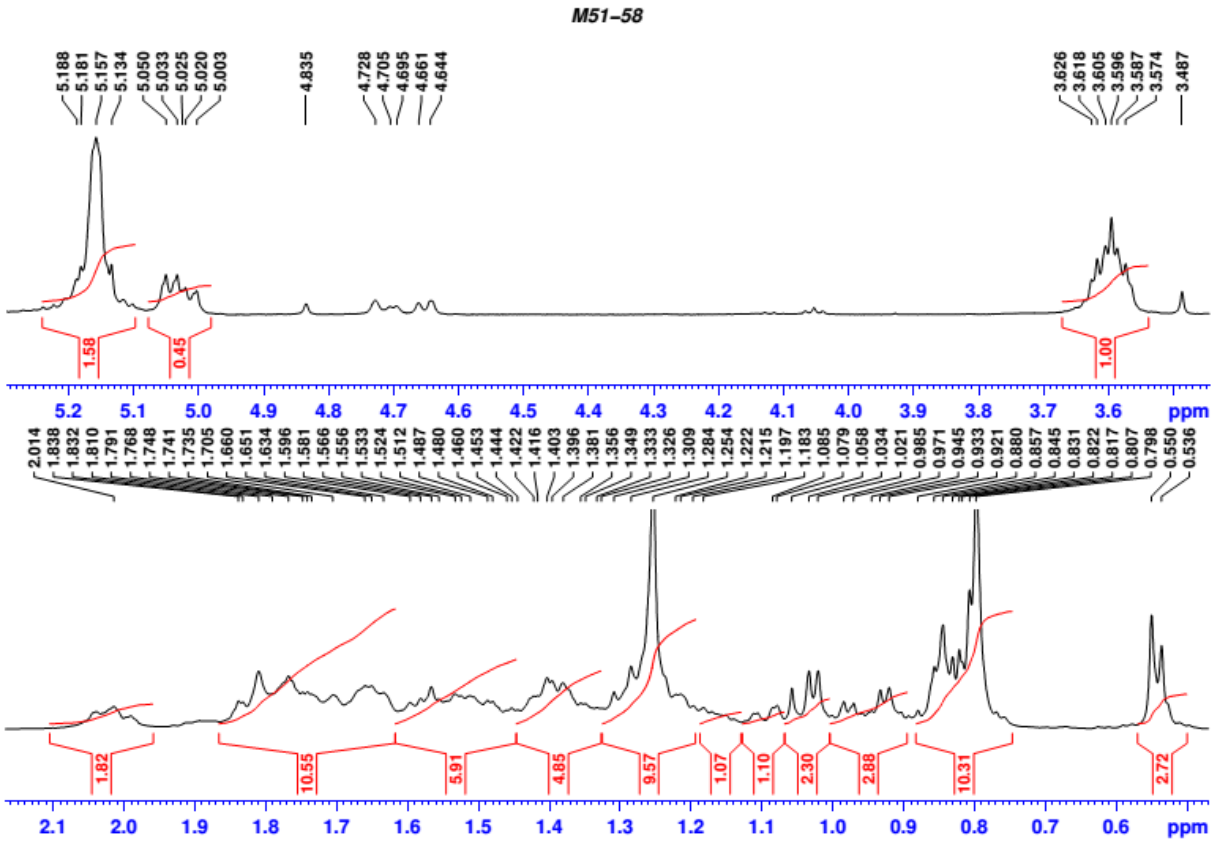

Figure 16: 125 MHZ ^{13} C NMR of Stigmasterol in CDC 13 of DMP

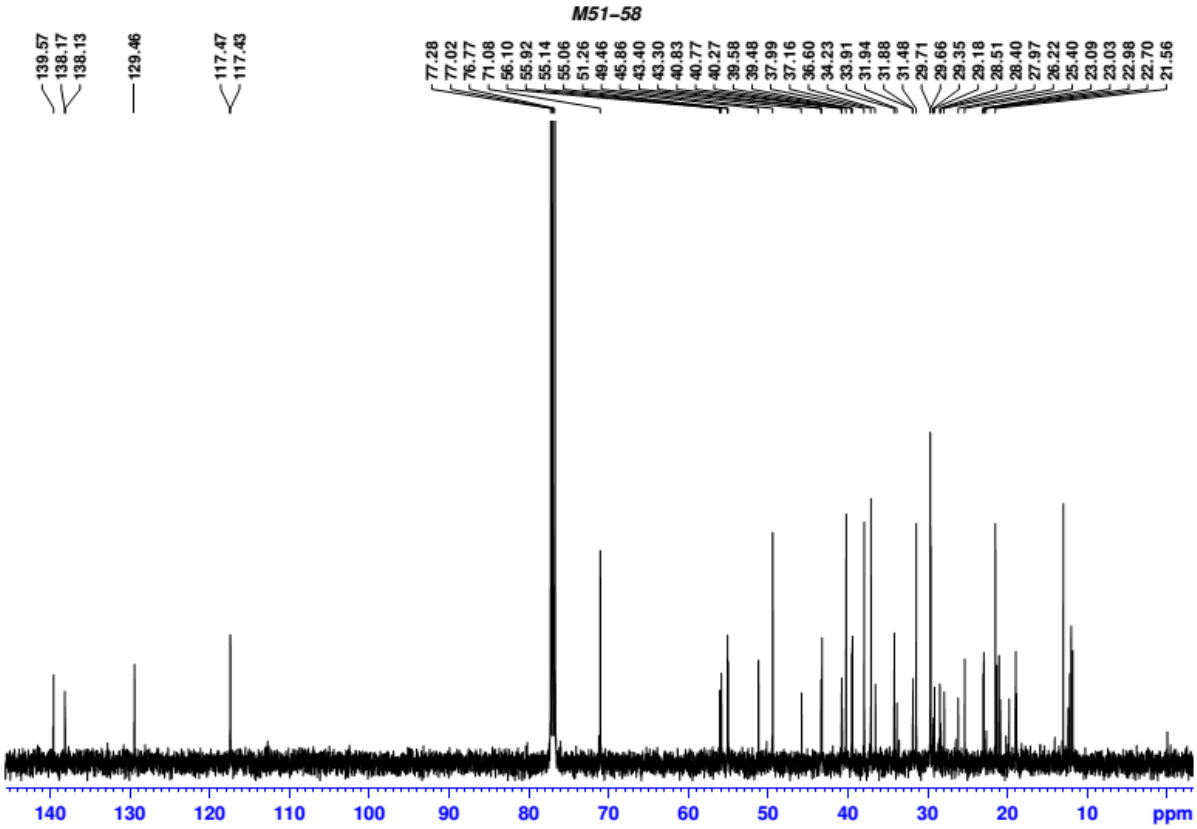

Figure 17: 125 MHZ ^{13} C NMR of Stigmasterol in CDC 13 of DMP

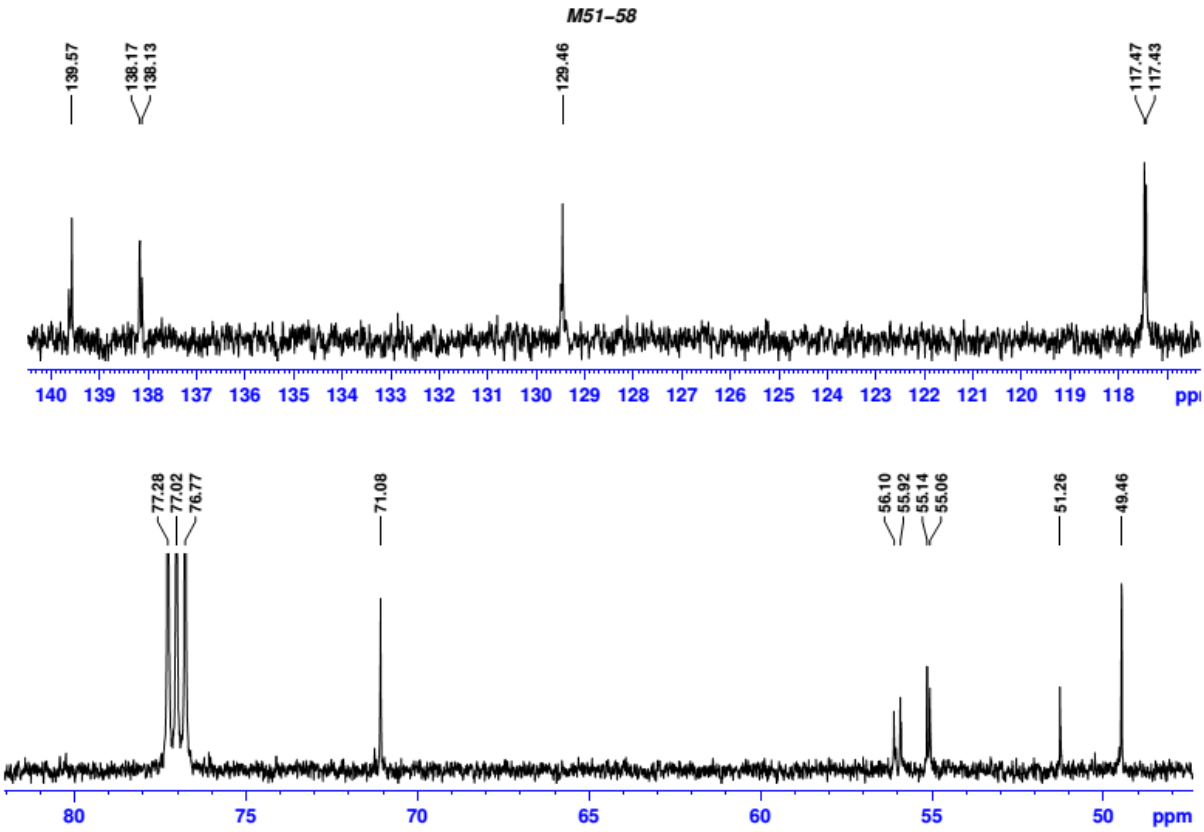

Figure 18: 125 MHZ ^{13} C NMR of Stigmasterol in CDC 13 of DMP

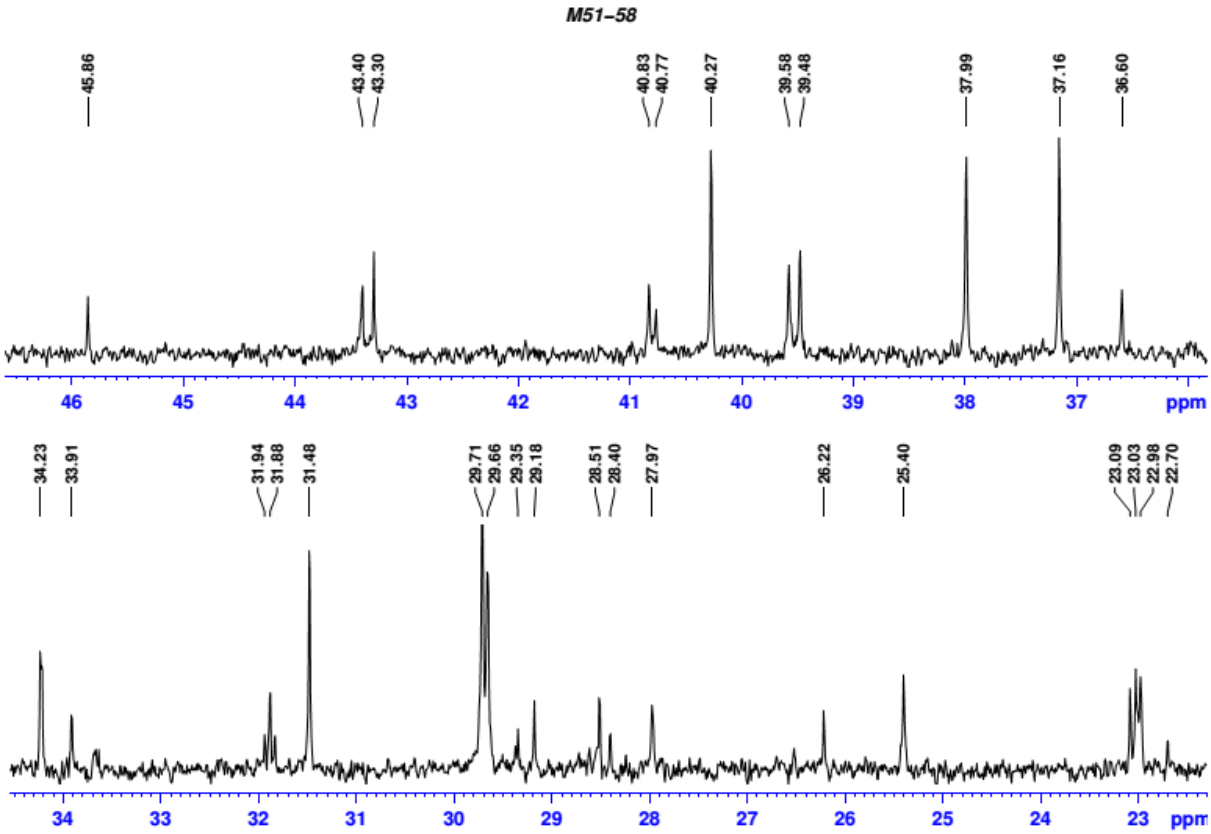

Figure 19: 125 MHZ ^{13} C NMR of Stigmasterol in CDC 13 of DMP

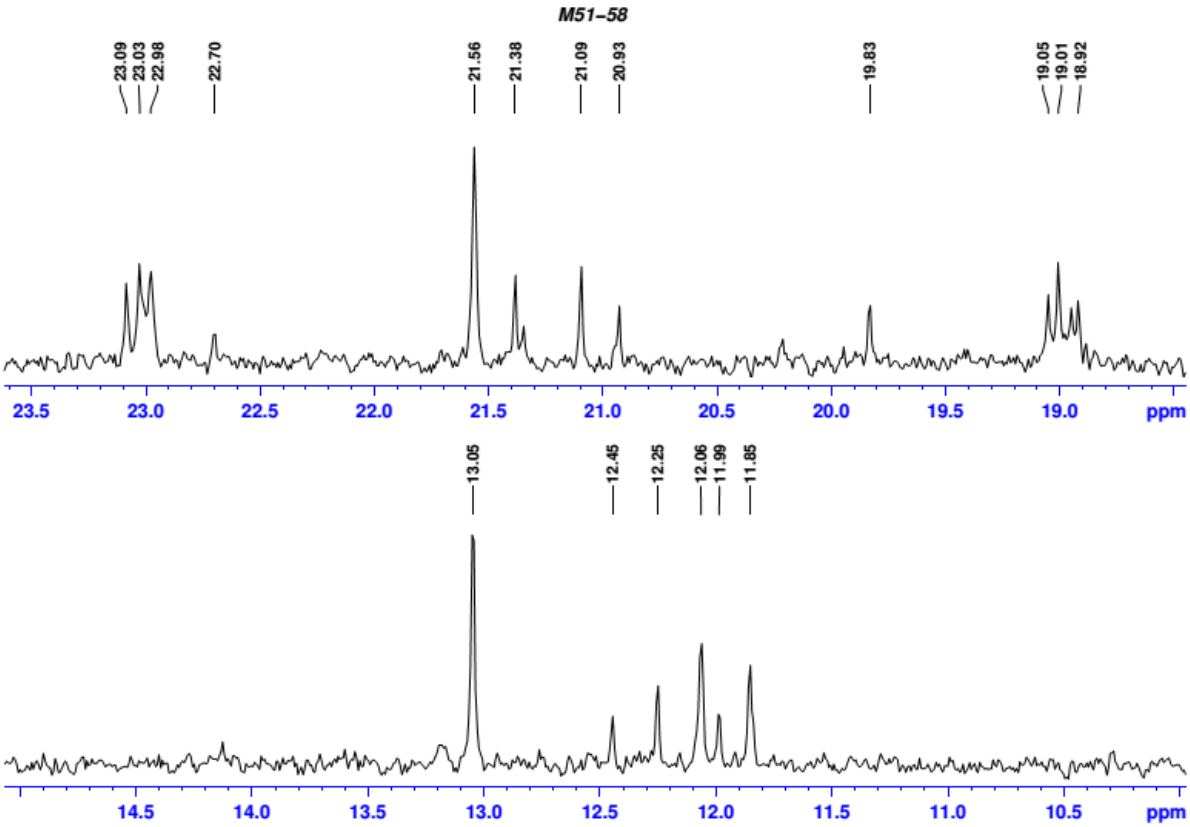

Figure 20: 125 MHZ ^{13} C NMR of Stigmasterol in CDC 13 of DMP

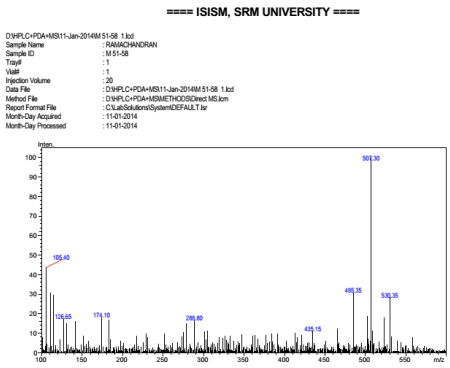

Figure 21: MASS spectra of DMP sample ID M51-58

Table 5: NMR Spectroscopic values of the compound Stigmasterol (51-58)

Position	Carbon signal(δ)	Proton signal(δ)	Position	Carbon signal(δ)	Proton signal(δ)
1	37.99		16	29.71	
2	31.48		17	49.46	
3	71.08	3.51	18	13.05	0.80
4	39.48		19	12.06	1.20
5	139.57		20	40.77	
6	117.43	5.2	21	21.38	
7	31.94		22	138.13	5.20
8	34.23		23	129.46	5.15
9	51.26		24	45.86	
10	37.16		25	25.40	
11	21.56		26	11.85	
12	40.27		27	29.66	
13	43.40		28	19.05	
14	55.92		29	19.01	
15	23.09				

RESULTS

The methanol extract of *Coldenia procumbens* Linn and *Mukia maderaspatana*(L) M. Roem was named as DCP and DMP and the % yield was calculated as 1.25. The colour of the extract was "Dark brown", stored in a desiccator [11, 12] .

In order to establish the relation between chemical content and chemo modulatory activity all phytodiversive chemicals and essential Flavonoids and phenol content of Dichloromethane, methanol, ethanol and water extracts of *Coldenia procumbens* Linn and *Mukia maderaspatana* (L) M. Roem was determined (Tables 2 and 3). Qualitative phytochemical analysis of Dichloromethane extract of *Coldenia procumbens* Linn and *Mukia maderaspatana* (L) M. Roem showed the presence of the majority of the compounds including alkaloids, glycosides, carbohydrates, steroids, sterols, flavonoids, tannins, proteins and amino acids. The phytochemical analysis of DCP and DMP results shown in Table 1. DCP plant contains alkaloids, carbohydrates, Flavonoids, Tannins, Protein and amino acid and DMP Plant contains alkaloids, carbohydrates, Flavonoids, Tannins, Phenol and Phytosterols. The results obtained showed that the extract process higher concentration of Flavonoids and phenol; the studies have shown that there is a clear relationship between total phenol and Flavonoid content and anticancer activity).

Acute Oral Toxicity Study results shown in Table 4. In this DCP and MCP were given to the animal at different concentration of 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg and the response like motor activity, pupils, urination, salivation, skin colour etc., are all normal when compared with the control group animals. And this study revealed the non-toxic potential of both the extracts tested.

Spectral analysis of the compound isolated from

Dichloromethane extract of Mukia maderaspatana, on subjection to I.R Spectroscopic analysis, the observed absorption bands were 3319cm^{-1} characteristic of O-H stretching. Absorption at 2929 cm^{-1} due to =CH str and 2863 cm^{-1} was assigned to C-H str. Other absorption frequencies included 1636 cm^{-1} as a result of C=C absorption; however, this band was weak, 1462 was a bending frequency for cyclic (CH2) n and 1382 cm^{-1} for –CH2 $(CH3)_2$.(Figure 4)

The Proton NMR has revealed the existence of signals for olefinic proton at δ5.2 and 5.15 (br.,s.), Angular methyl proton at 0.80 (s), and 1.2(s) corresponding to C18 and C19 proton respectively (Table 5Figure 5).

The ^{13}CNMR has shown recognizable signals δ 139.5 and 117.43, which were assigned C5 and C6 double bonds respectively. The δ value at δ 71.0 is due to C.3 β hydroxy group. It was complemented by the presence of a multiplet signal at δ 3.51 in the ^{1}H-NMR.

The rest of the ^{13}CNMR signals closely resemble the signals of Stigmasterol (Table-5)Spectral analysis of the compound isolated from Dichloromethane extract of Coldenia procumbenson subjection, the IR spectrum exhibited characteristic absorption bands at 1735 cm^{-1} for ester carbonyl group and at 1169 cm^{-1} for C-O group (Figures 6, 7, 8, 9 and 10).

The ^{1}H-NMR exhibited a triplet at δ 4.06 (2H, J= 7.0 Hz) characteristic of a -CH_2-O- group, a triplet at δ 2.35 for a -CH_2-CO- group, a triplet at δ 0.86 (3H, J= 7.0 Hz) for a methyl group, a strong singlet at δ 1.29 characteristic of a long chain methylene group.

The ^{13}C-NMR spectra exhibited characteristic signal at δ 174.08 (C=O), 64.43 (-CH_2-O), 14.12 and 22.71 for two methyl groups, and a bunch of signals centred at δ 29.38 characteristic of a long chain methylene carbons (Figures 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20).

The molecular weight of the compound was 410 (Molecular ion peak at m/z 410 and a peak at m/z 409 for [M-H]$^{+}$ ion). The above data suggested that the compound may be a long chain fatty ester (Figure 21).

Spectral analysis of the compounds isolated from Dichloromethane extract of *Mukia maderaspatana* and *Coldenia procumbens*, on subjection to I.R Spectroscopic analysis, ^{1}H-NMR,^{13}C-NMR spectra and molecular weight of the compound revealed the presence of Stigmasterol and a long-chain fatty ester.

CONCLUSION

With the above said findings, we conclude that the plant *Coldenia procumbens* Linn and *Mukia maderaspatana* (L) M. Roem posses chemical compounds that show antioxidant and anticancer activity. *Mukia maderaspatana* (L) M. Roem, *Coldenia procumbens* linn before its clinical usage, through toxicological profile was determined on the crude extracts as well as on isolated compounds to confirm the safety of the drug.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

FUNDING SUPPORT

None

ACKNOWLEDGEMENT

The authors are thankful to all who have extended their constant support for the completion of the work.

REFERENCES

[1] Asmara AP. A preliminary study of investigating of compound group contained in ethanolic extract of mahagony (Swietenia mahagoni L. Jacq.) seeds related to α-glucosidase inhibition. Jurnal Natural. 2018;18(2).

[2] West BJ, Deng S. Thin-layer chromatography methods for rapid identity testing of Morinda citrifolia L. (Noni) fruit and leaf. Advance Journal of Food Science and Technology. 2010;2(5):298–302.

[3] Schrage A, Hempel K, Schulz M, Kolle SN, van Ravenzwaay B, Landsiedel R. Refinement and Reduction of Acute Oral Toxicity Testing: A Critical Review of the Use of Cytotoxicity Data. Alternatives to Laboratory Animals. 2011;39(3):273–295. Available from: 10.1177/026119291103900311.

[4] Schlede E, Genschow E, Spielmann H, Stropp G, Kayser D. Oral acute toxic class method: A successful alternative to the oral LD50 test. Regulatory Toxicology and Pharmacology. 2005;42(1):15–23. Available from: 10.1016/j.yrtph.2004.12.006.

[5] Ojer P, de Cerain AL, Areses P, Peñuelas I, Irache JM. Toxicity Studies of Poly(Anhydride) Nanoparticles as Carriers for Oral Drug Delivery. Pharmaceutical Research.

2012;29(9):2615–2627. Available from: 10.1007/s11095-012-0791-8.

[6] Symeou S, Tsiafoulis CG, Gerothanassis IP, Miltiadou D, Tzamaloukas O. Nuclear magnetic resonance screening of changes in fatty acid and cholesterol content of ovine milk induced by ensiled olive cake inclusion in Chios sheep diets. Small Ruminant Research. 2019;177:111–116. Available from: 10.1016/j.smallrumres.2019.06.017.

[7] Das S, Singh S, Sharma V, Soni ML. Biotechnological applications of industrially relevant amylase enzyme. In International Journal of Pharma and Bio Sciences. 2011;2(4):166–175.

[8] Gerothanassis IP, Troganis A, Exarchou V, Barbarossou K. Nuclear Magnetic Resonance (NMR) Sectroscopy: Basic principles and phenomena, and their applications to biochemistry, biology and medicine. Chem Educ Res Pract. 2002;3(2):229–252. Available from: 10.1039/b2rp90018a.

[9] Tsiafoulis CG, Skarlas T, Tzamaloukas O, Miltiadou D, Gerothanassis IP. Direct nuclear magnetic resonance identification and quantification of geometric isomers of conjugated linoleic acid in milk lipid fraction without derivatization steps: Overcoming sensitivity and resolution barriers. Analytica Chimica Acta. 2014;821:62–71. Available from: 10.1016/j.aca.2014.03.010.

[10] Hoskinson AR, Singh PK, Sonkusale S, Hopwood J. Low-Voltage Switchable Microplasma Arrays Generated Using Microwave Resonators. IEEE Electron Device Letters. 2013;34(6):804–806. Available from: 10.1109/led.2013.2257659.

[11] Ramachandran K, Venketnarayanan R. Antioxidant properties of the extract of whole plant parts of coldenia procumbens. International journal of phytopharmacology. 2019;10(2):44–57.

[12] Ramachandran K, Venketnarayanan R. Antioxidant Potential of the extract of whole plant parts of Mukia maderaspatana (linn.)Roem. International Journal of Biological & Pharmaceutical Research. 2019;10(1):1–14.

ABOUT AUTHORS

Ramachandran K

Cite this article: K Ramachandran, R Venketnarayanan, Rajesh Yadav, R Suresh, Sumitra Devkota. **Phytochemical screening and acute oral toxicity study of *Coldenia procumbens* and *Mukia maderaspatana* whole-plant extracts**. Int. J Nov. Tren. Pharm. Sci. 2019; 9(2): 16-31.