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ISOLATION AND CHARACTERIZATION OF NATURAL COMPOUNDS FROM LIQUORICE

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Abstract:

The present research work is mainly concened with the natural compounds that are obtained from traditional medicinal plants. There are number of natural compounds have been available in the nature. Some natural compounds are very useful to human being and have life pontential to save human from many uncurable disease. Keeping view in the mind the research is focused to extract and isolate antioxidant, anti-inflammatory, anticancer, antihyperlipidmic, antidiabetic, antiulcer activites containing compounds are studied. The major natural compounds are liquorice, ginger, alliuam, triphala (harda, bahera, pipali, amla and guggulu). The glycyrrhyza glabra is obtained from rhizomes of liquorice and useful as expectorant and ulcer healing properties with carbenoloxolone as major constituents. All components are isolated from the concerned extracts. The extractions of all plants are based on successive solvent extraction method for all drugs. The constituents are confirmed by structure elucidation. The structure of each compounds are intrepereted by different spectral techniques like Infrared spectrum, nuclear magnetic spectrum (hydrogen anc carbon thirteen spectra) and mass spectroscopy for molecural formula and molecular weight of the unknown compounds.

Key words: *Liquorice, extraction and* ***chromatography***

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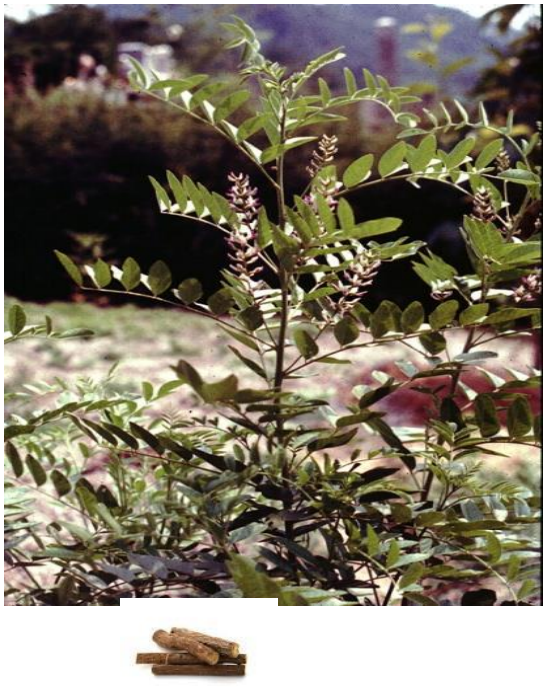
INTRODUTION:

Natural compounds

Natural compounds are obtained in the nature from different medicinal and herbal plants. These are generally secondary metabolites of the various plant parts or waste compound of plant. These are obtained various parts of the herbs, shrubs or plants like stem, root, leaves, fruits, seeds, barks, rhizomes, flowers and entire plants. The particular part of the plant is collected, extracted and isolated for the collection of various types of natural compounds. We include alkaloids, glycosides, terpenoids, resins, tannins, carbohydrates, gums and exudates, liganans. These obtained drugs have various types of pharmacological and therapeutic application[1-6].

Liquorice (Glycyrrhiza, Mulethi): liquorice consists of dried, peeled or unpleed, root & stolon of Glycyrhiza glabara belonging to family Leguminosae. It is also obtained from other species of glycyrrhiza, giving a drug with sweet taste. Liquorice have water soluble extractive not less than 20% w/w. On addision of 80% sulphirc acid, the thick secsion of drug or powder shows deep yellow color. Liquorice owes most of its sweet taste to glycyrhine, the potassium & calcium salts of glycyrhineic acid. The yellow color of liquorice is due to flavonoids. Carbenoloxone is used for ulcer healing as protective base[7-16].



***Glycyrrhiza Glabra* Plant; Inset: Rhizome and roots**

Taxonomy of Plant (Liquorice)

Botanical Name – *Glycyrhiza glabara*

Kingdom -	Planate – Plants
Subkingdom -	Tracheobionta - Vascular plants
Super division -	Spermatophyta - Seed plants
Genus -	*Glycyrhiza* - Liqorice
Division -	Magnoliophyta - Flowering plants
Subclass -	Rosidae
Order -	Fabales
Family -	Leguminosae/Fabaceae - Pea Family
Species -	*Glycyrhiza glabara* L. Cultivated Liqorice

Common Name – Liquorice, Liqorice (English), Lacrisse (German), Reglisse (French), Regolizia (Italian), Kanzoh (Japanese), Gancao (Chinese), Yasti-madhu in Ayurveda & Mulethi (Hindi).

Phytochemistry:

Major Saponin: Glycyrrhizic acid is the major triterpenoid saponin (4–20%) in Liqorice rhizome & is used as a tool for recognizing the herb.

Minor Sapogenins: About fifty other sapogenins have been isolated from *Glycyrhiza* species. *G. glabra* have 13 minor sapogenins, liquoric acid is found in high amount as compare to other minor sapogenins, these are glabrolide, 11-deoxyglabrolide, liquiritic acid, isoglabrolide, 11-deoxoglycyrrhitinic acid, glycyrretol, 24-hydroxyglycyrrhetic acid, 24-hydroxy-11-deoxoglycyrrhitic acid, liquiridiolic acid etc [17-25].

EXPERIMENTAL:

General

Pure marker compounds were isolated by using various chromatographic & their structures were determined based on various spectroscopic techniques as mentioned below.

Thin layer chromatography: Chromatographic reactions were monitored on analytical TLC (MERCK TLC Silica gel 60 F_{254}) precoated plates. TLC plates were developed in CAMAG glass twin trough chamber (20 × 10 cm). TLC chromatograms were visualized by: (a) UV Detection Chamber at 254nm, (b) UV Detection Chamber at 366 nm (CAMAG) & (c) derivatizing TLC plats with 0.5% anisaldehyde in 5% sulphuric acid & charring them at high temperature (80-100 ^{0}C) in a hot air oven.

Column chromatography: Column chromatography was performed by using silica gel (60-120 mesh), followed by purification with silica gel (100-200 or 200-400 mesh) column or with Sephadex LH-20 (size exclusion chromatography).

Spectroscopic techniques: For establishing the chemical structure of pure marker compounds & their derivatives, ^{1}H NMR, ^{13}C NMR, DEPT & COSY experiments were performed on the BRUKER AVANCE 200, 400, & 500 MHz instrument with tetra methyl silane (TMS) as an internal standard. Chemical shift was given in δ-ppm value. Electro spray mass (ES–MS) spectra were recorded on HP-1100 MSD instrument.

Dictionary of Natural Compounds: (CRC, Chapman & Hall, London, 2011) isolated compounds were dereplicated & identified based on their report available in DNP & some compounds were identified by comparison (TLC, CO-TLC, ^{1}H-NMR, MS) with authentic samples[26-28].

Plant material

The rhizome of *Glycyrhiza glabara* plant was collected from botany department of Indian Institute of Integrated Medicine (IIIM), Jammu. The plant was identified & authenticated by botanist, Dr. S.N. Sharma, Department of Taxonomy, IIIM, and Jammu. A voucher specimen is held in the institutional Herbarium.

Extraction & Isolation of Marker compounds from *G. glabra*

Extraction: 1 kg Powdered rhizomes were extracted with 1 L ethanol with mechanical stirring at room temperature this process repeated for 4 times for complete extraction. Then this extract was filtered & concentrated under vacuum (175 mbar) at 40 ^{0} C by using Rota vapor to provide crude extract (300 g).

Isolation of marker compounds: A neat & dried glass column was taken. A cotton plug was put at the base of the column & packed with silica gel (60-120 mesh, 1100 g).Then the extract (250 g) was dissolved in minimum quantity of chloroform-methanol & it was mixed with 500 g silica gel (60-120 mesh) for slurry & charged into the column.

Column Specifications

Column Diameter	10 cm
Length of column	150 cm
Silica gel (60-120)	1100 g
Bed Length	35 cm

The column was eluted with step gradient solvent system of hexane-chloroform-ethyl acetate-methanol & water & 215 fractions were collected (100 ml each). All fractions were pooled on the basis of TLC & divided into 5 parts (Hexane fr-1 to 10, Chloroform fr-11 to 80, Ethyl acetate fr-81 to 180, Methanol fr-181 to 210 & Water fr-211 to 215) & then concentrated under high vacuum. All the fractions were then individually subjected to Column chromatography (Silica Gel 100-200 mesh) & Sephadex (LH-20) to isolate pure compounds as represented[29-35]

Identification of isolated compounds was carried out by comparison of spectral data & physical data with the reported data.

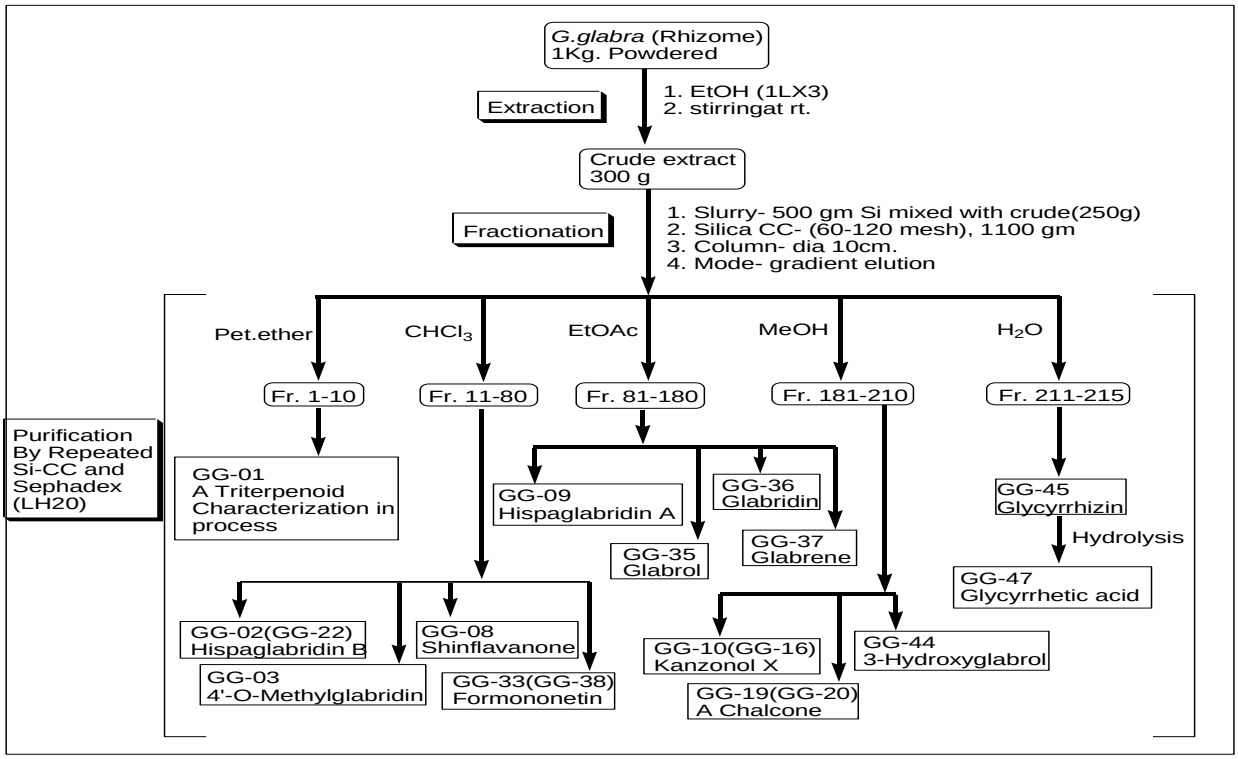
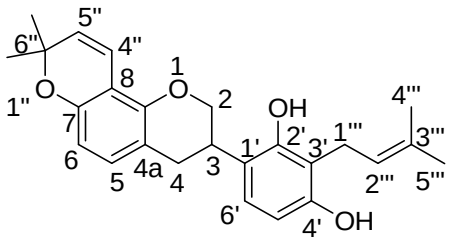


Fig1: Extraction & Isolation Protocol.

RESULTS & DISCUSSION:

Characterization of marker compound.

1. **Compound GG-09 (Hispaglabridin A)**



Structure of Hispaglabridin-A

Colourless needle shaped crystalline compound (25 mg) isolated from ethyl acetate fraction using silica gel (100-200 mesh) column chrometography& Sephadex (LH-20). It was eluted in 50% of ethyl acetate in hexane from silica gel column & then purified by LH-20 using methanol as eluting solvent. It was then recrystallized using cyclohexane. The structure of compound GG-09 was characterized as Hispaglabridin by comparison of its spectral data with reported data.

TLC: R_F= 0.40, Hexane: Ethyl acetate (30: 70), Visualization: UV-254 nm.

M.P. = 130-135 ^{0}C

^{1}H- NMR (500 MHz, CDCl3):δ(ppm) 1.45 (6H, br s, $(CH_3)_2$-6"), 1.78 (3H, s, CH_3 -5'''), 1.84 (3H, s, H-4'''), 2.85 (1H, dd, *J* = 3.5 Hz, H-2), 2.96 (1H, dd, *J* = 10 Hz, H-2), 3.40-3.47 (1H, m, H-3), 3.49 (2H, br d, *J* = 7 Hz, H-1'''), 4.02 (1H, t, *J* = 10.5 Hz, H-4), 4.34 (1H, br dd, *J* = 10 Hz, H-4), 5.25 (1H, s, OH-2'), 5.27 (1H, br t, *J* = 6.5 Hz, H-2'''), 5.59 (1H, d, *J* = 10 Hz, H-5'), 6.28 (1H, d, *J* = 8 Hz, H-6), 6.39 (1H, d, *J* = 8 Hz, H-5), 6.64 (1H, d, *J* = 9.9 Hz, H-4"), 6.79 (2H, d, *J* = 8 Hz, H-5", 6").

13C-NMR (400 MHz, CDCl3): 17.8 (C-5'''), 22.8 (C-1'''), 25.8 (C-4'''), 27.6 (C-6a"), 27.7 (C-6b"), 31.0 (C-4), 31.7 (C-3), 70.1 (C-2), 75.9 (C-6"), 107.4 (C-6), 108.0 (C-5'), 109.5 (C-4a), 114.3 (C-8), 114.6 (C-3'), 116.6 (C-5"), 121.4 (C-1'), 122.1 (C-2'''), 126.9 (C-5), 127.6 (C-4"), 129.2 (C-6'), 134.2 (C-3'''), 150.2 (C-7), 151.5 (C-4'), 152.4 (C-2'), 153.6 (C-8a).

MS-ES: (Negative) m/z 391 $[M-H]^+$ so the mass of the compound m/z 392 $[M]^+$corresponded to molecular formula (MW.-392.13).

^{1}H NMR

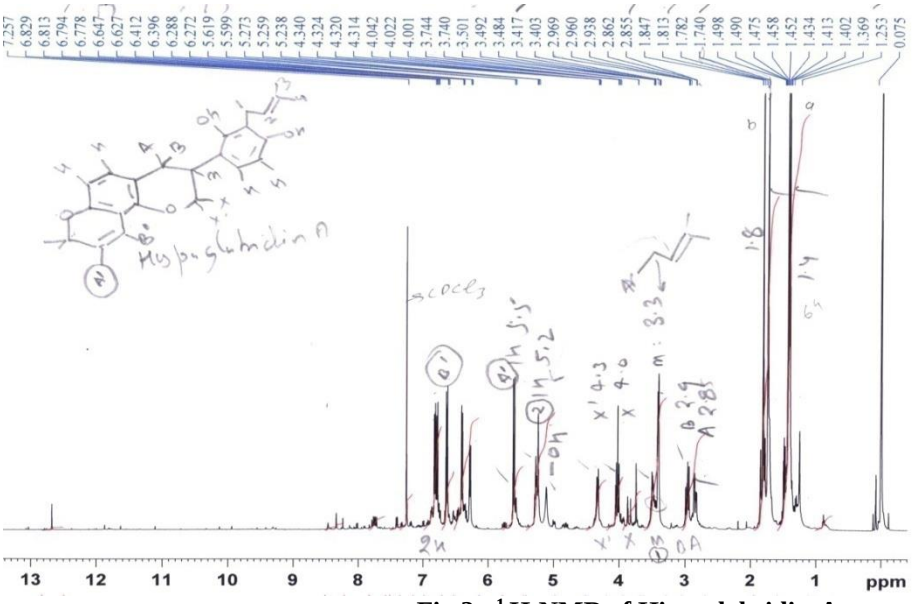


Fig 2: ^{1}H-NMR of Hispaglabridin-A

Name of atom	Chemical shift	Interpretation
Ar hydrogen	6-8.2 p.p.m	neighboring group effect
Aliphatic hydrogen	2-3.2 ppm	splitting of signal
Vinyl group hydrogen	3.5-4 ppm	intensity is fast
Hydroxyl group hydrogen	2.1-2.5 ppm	number of signal
Epoxide hydrogen	2.3-2.4 ppm	nil
Methyl group	1.2-1.5 p.p.m	primary hydrogen

On the basis of hydrogen nuclear magnetic spectral the compound has aromatic and aliphatic hydrogen that are identified on the basis of chemical shift value in parts per million. The vinyl hydrogen has different chemical shift value. The methyl group contains primary hydrogen with minimum chemical shift value in parts per million. The epoxide shows different part per million ppm value of chemical shift.

GG-09 (Hispaglabridin A)

^{13}C NMR

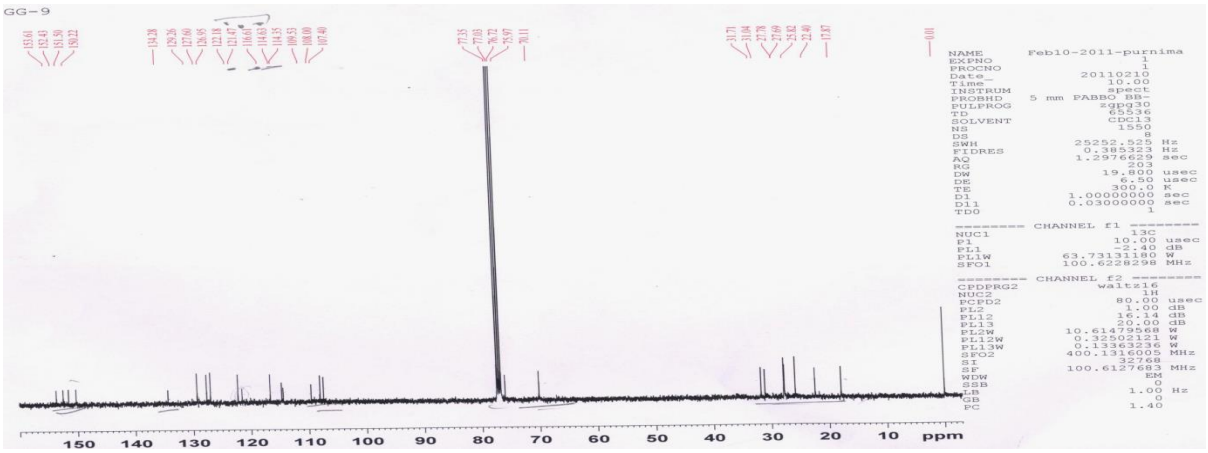


Fig 3: ^{13}C-NMR of Hispaglabridin-A

Name of atoms	Chemical shift	Interpretation
Aromatic carbons	26-28.3 parts per million	neighboring group effect
Aliphatic carbons	22-23.2 ppm	splitting of signal
Vinyl group carbon	2 3.5-4 ppm	intensity is fast
Hydroxyl group hydrogen	20.1-22.5 ppm	number of signal
Epoxide carbon	20.3-20.4 ppm	opposed
Methyl group	21.2-21.5 ppm	primary carbon

On the basis of C^{13} nuclear magnetic resonsnce spectra (NMR) the compound has aromatic and aliphatic carbon that are identified on the basis of chemical shift value in parts per million. The vinyl hydrogen has different chemical shift value. The methyl group contains primary carbon with minimum chemical shift value in parts per million. The epoxide shows different part per million value of chemical shift.

GG-09 (Hispaglabridin A)

DEPT (135)

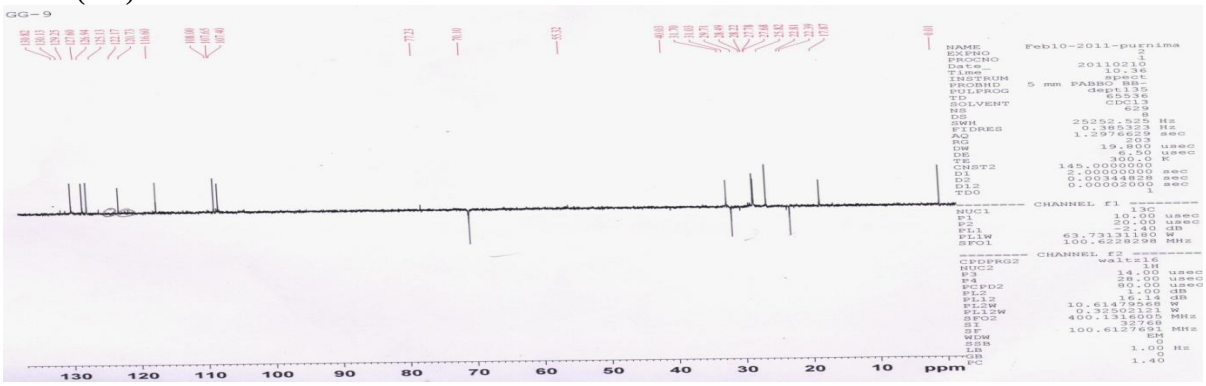


Fig 4: DEPT of Hispaglabridin-A

Name of atom	Chemical shift	Interpretation
Aromatic deuterium	6-8.5 ppm	neighboring group effect
Aliphatic deuterium	2-3.2 ppm	splitting of signal
Vinyl group deuterium	3.5-4 ppm	intensity is fast
Hydroxyl group deuterium	2.1-2.5 ppm	number of signal
Epoxide deuterium	2.3-2.4 ppm	nil
Methyl group	1.2-1.5 ppm	primarydeuterium

On the basis of deuterium 2 nuclear magnetic resonance spectrum **(^{2}D N M R)** the compound has aromatic and aliphaticdeuterium that are identified on the basis of chemical shift value in parts per million. The vinyldeuterium has different chemical shift value. The methyl group contains primarydeuterium with minimum chemical shift value in parts per million. The epoxide shows different part per million values of chemical shift.

GG-09 (Hispaglabridin A)

ES-MS

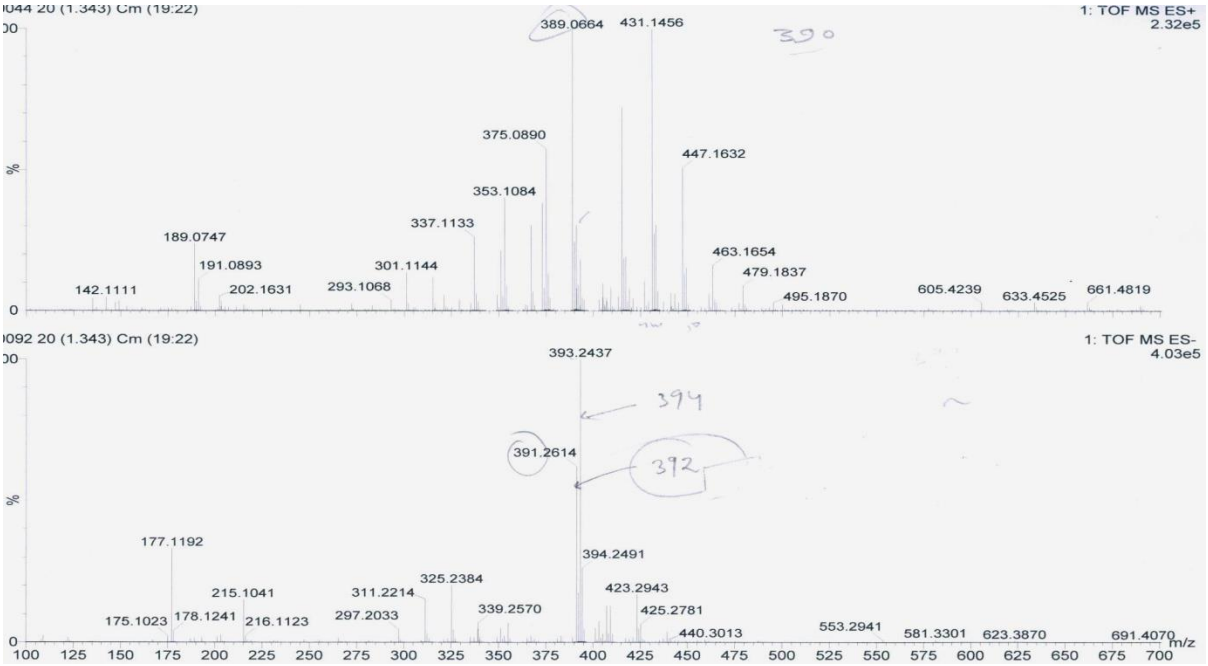
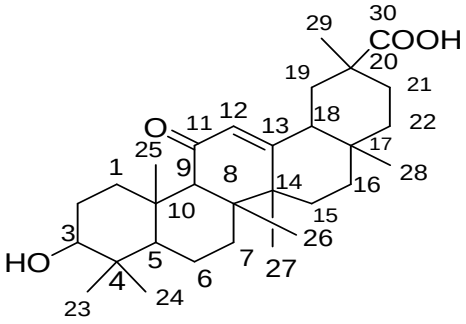


Fig 5: ES-MS of Hispaglabridin-A

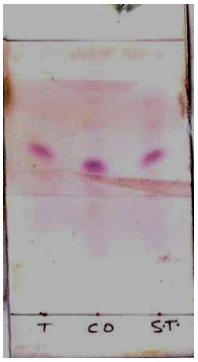
Mass Spectrum of Hispaglabridin A

On the given spectra of mol. mass we can determine the molecular wt of the compound. We know that the molecular ion peak gives the mass or molecular weight of the uknown compounds. In the given figure the base peak shows the peak at 394.876 that is the mass of the product. The parent peak is known as mass or molecular weight of the compound. The fragementations of the compounds shows breaking points and metabolic products of the compounds that is very helpful for structure elucidation of the compounds. The m+1, m+2, m+3 etc. Peaks are known as isotopic peak of the compound. These peaks are very helpful to determine the molecular fornula of the unknown compounds. These two things molecular weight and molecular formular are very important determinant of the structural elucidation of the unkonw compound. We represent relative intensity or abundance on the y-axis and molecular mass on the x-axis for interpretation[36-43].

12. Compound GG-47 (Glycyrrhetic acid)



Structure of Glycyrretic acid



1. T: Test Compound; 2. Co: Co-Spotting; 3. ST: Standard

Derivatized with AS reagent

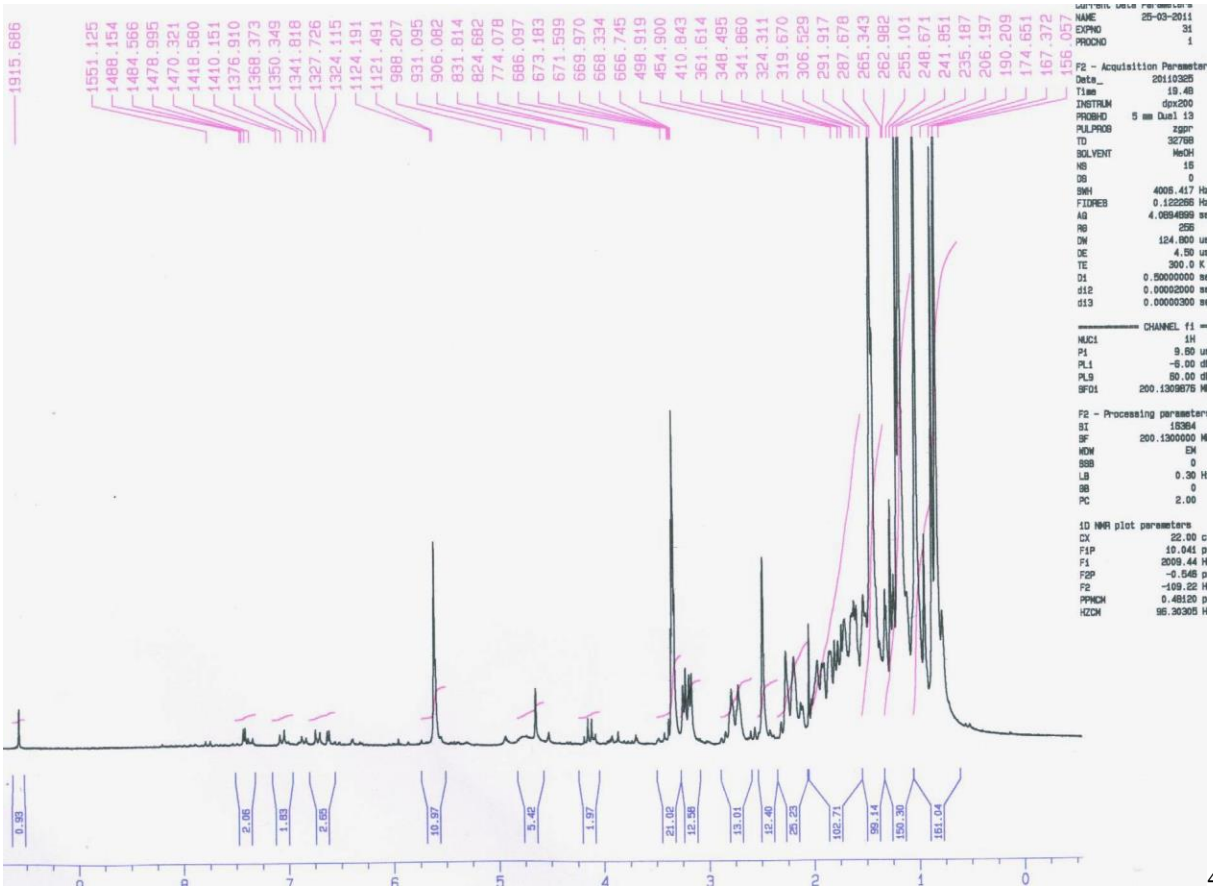
100 mg Glycyrhine was dissolved in 5 ml methanol & it was added to 5% HCl in a 50 ml round bottle flask. The reaction mixture was refluxed for 24 h. at 100 ^{0}C. Reaction was monitored by TLC, after completion of reaction, it was concentrated to evaporate methanol & portioned with ethyl acetate (50 ml x 3), ethyl acetate portion was dried over sodium sulfate, & concentrated to get 50 mg colorless amorphous compound GG-47 which was identified as Glycyrrhetic acid by H-NMR & Mass spectroscopy.

^{1}H NMR (500 MHz, MeOD): δ(ppm) 0.72 (3H, s, H-3), 0.78 (3H, s, H-3),0.91 (3H, s, H-3), 1.02 (6H, s, H-3),1.06 (3H, s, H),1.31 (3H, s, H-29),3.16 (1H, dd, *J* = 11.8,4.2 Hz, H-3), 5.58 (1H, s, H-12).

MS-ES: At (Negative) modem/z 469 [M-H] $^+$& in (Positive) mode the m/z 471 [M+H] $^+$ so the m/z 470 [M] $^+$ corresponded with molecular formula C_{30} H_{46} O_4 (MW.- 470.32).

GG-47 (Glycyrrhetic acid)

^{1}H NMR



4.3

Fig 6: ^{1}H-NMR of Glycyrhtic acid

Name of atom	Chemical shift	Interpretation
Vinyl group hydrogen	3.5-4 ppm	intensity is fast
Hydroxyl group hydrogen	2.1-2.5 ppm	number of signal
Methyl group	1.2-1.5 ppm	primary hydrogen

On the basis of hydrogen nuclear magnetic resonance NMR spectrum the compound has aromatic and aliphatic hydrogen that are identified on the basis of chemical shift value in parts per million. The vinyl hydrogen has different chemical shift value. The methyl group contains primary hydrogen with minimum chemical shift value in parts per million. The epoxide shows different part per million values of chemical shift.

GG-47 (Glycyrrhetic acid)

ES-MS

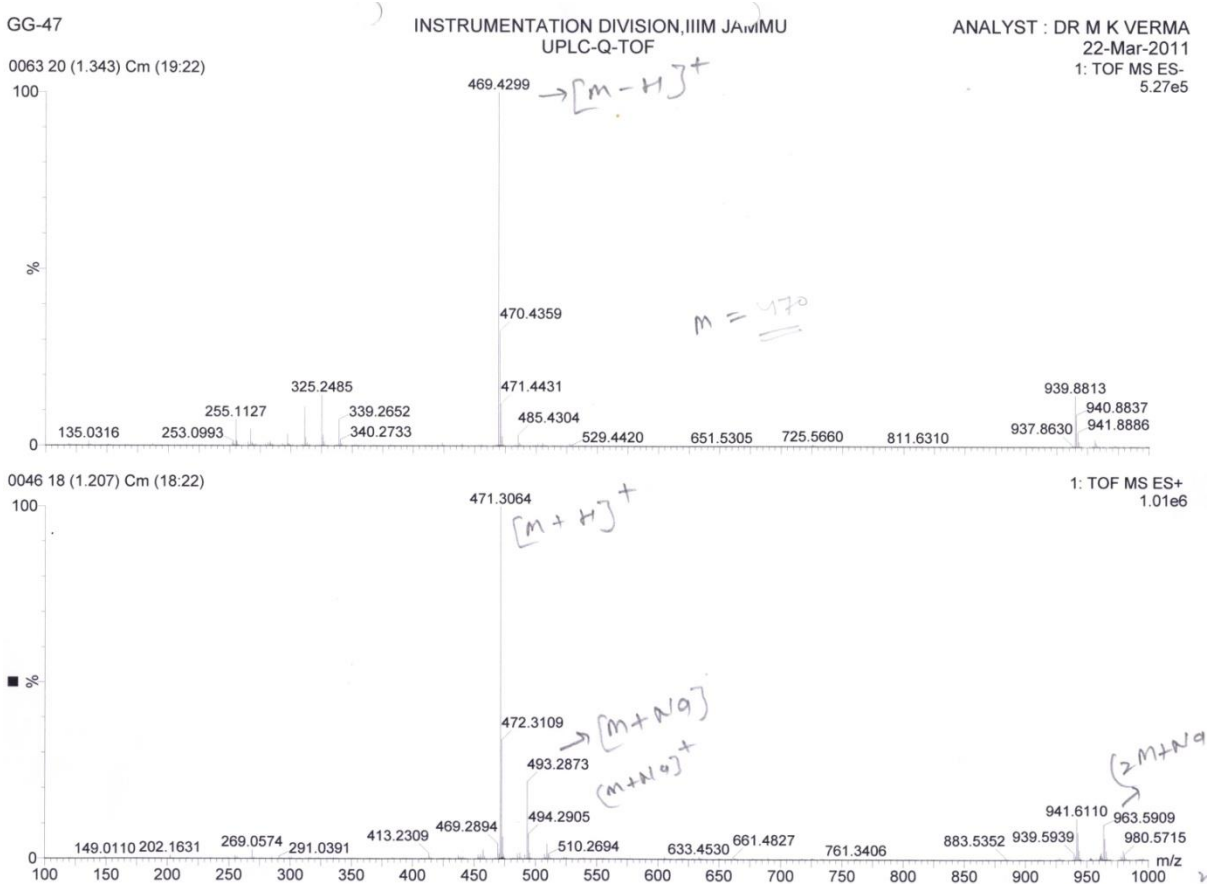
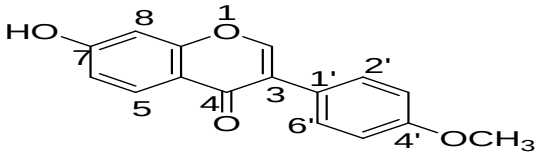


Fig 7: ES-MS of Glycyrhtic acid

Mass Spectra of Glycyrryhyzic acid

Basis of molecular mass we can determine the molecular weight of unknown of the compound. We know that the molecular ion peak provides the mass or molecular weight of the uknown compounds. In the given figure the base peak shows the peak at 587.143 that is the mass of the product. The parent peak is known as mass or molecular weight of the compound. The fragementations of the compounds show breaking points and metabolites of the compounds that is very helpful for structure elucidation of the compounds. The m+1, m+2, m+3 etc. Peaks are known as isotopic peak of the compound. These peaks are very helpful to determine the molecular fornula of the unknown compounds. These two things molecular weight & molecular formular are very important determinant of the structural elucidation of the unkonw compound. We represent relative intensity or abundance on the y-axis and molecular mass on the x-axis for interpretation.

GG-33 (Formononetin)



^{1}H NMR

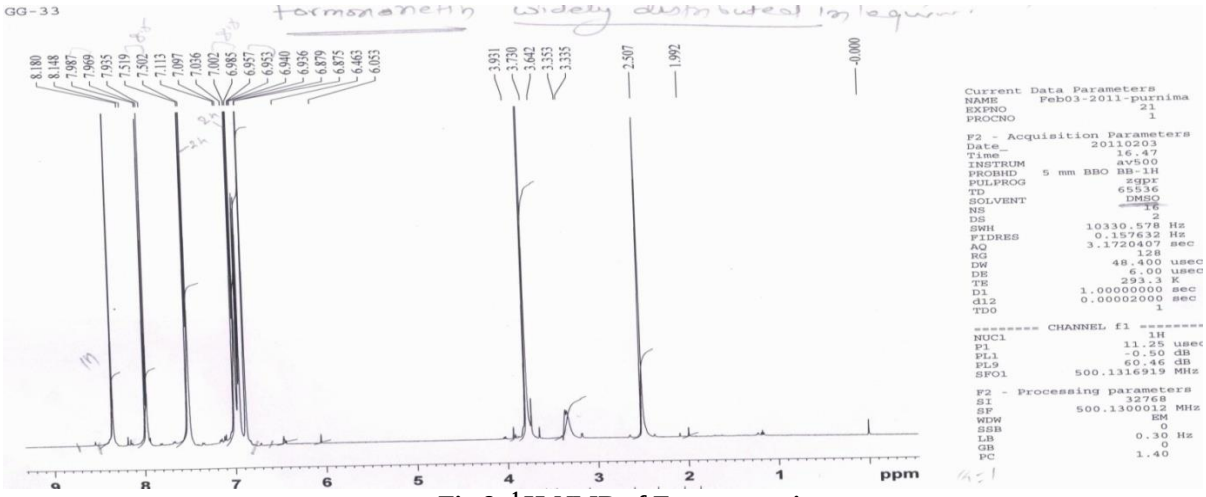


Fig 8: ^{1}H-NMR of Formononetin

Name of atom	Chemical shift	Interpretation
-C6H3- hydrogen	6-8.5 ppm	neighboring group effect
Aliphatic hydrogen	2-3.2 p.p.m	splitting of signal
Vinyl group hydrogen	3.5-4 ppm	intensity is fast
Hydroxyl group hydrogen	2.1-2.5 p.p.m	number of signal
Epoxide hydrogen	2.3-2.4 ppm	nil
Methyl group	1.2-1.5 ppm	primary hydrogen

On the premise of 2D atomic attractive range (2D NMR) the compound has sweet-smelling and aliphaticdeuterium that are distinguished on the premise of substance move an incentive in parts for each million. The vinyldeuterium has distinctive ^{1}H NMR (Expention) substance move esteem. The methyl assemble contains primarydeuterium with least synthetic move an incentive in parts for every million. The epoxide indicates diverse part per million estimation of compound move.

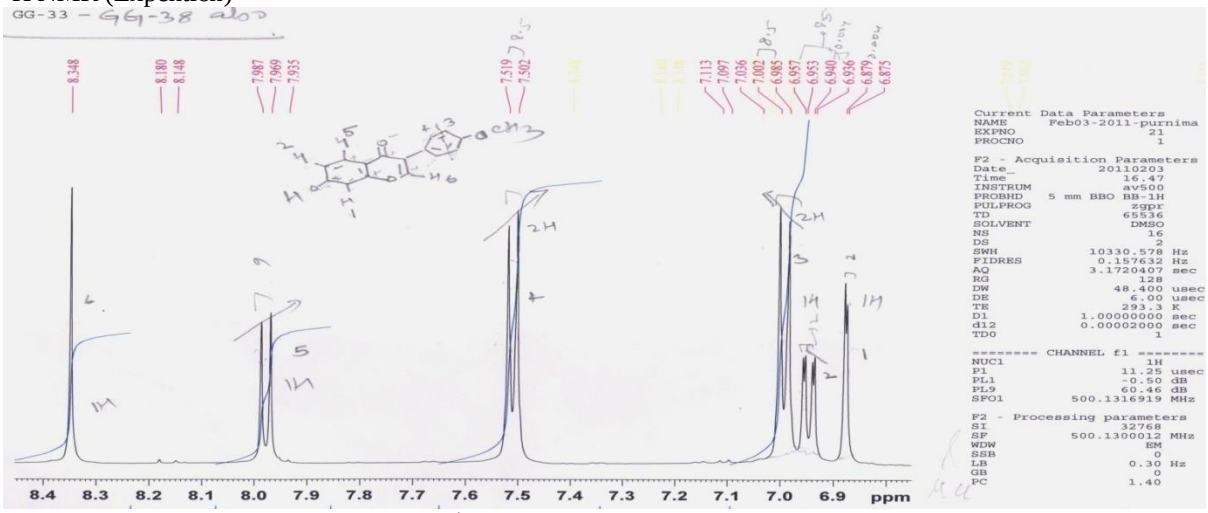


Fig 9: ^{1}H-NMR(exaption) of Formononetin

Name of atom	Chemical shift	Interpretation
Aroma. hydrogen	6-8.5 p.p.m	neighboring group effect
Aliphatic hydrogen	2-3.2 ppm	splitting of signal
Vinyl group hydrogen	3.5-4 ppm	intensity is fast
Hydroxyl group hydrogen	2.1-2.5 ppm	number of signal
Epoxide hydrogen	2.3-2.4 ppm	nearby proton
Methyl group	1.2-1.5 ppm	primary hydrogen

On the premise of hydrogen atomic attractive spectra the compound has fragrant and aliphatic hydrogen that are distinguished on the premise of concoction move an incentive in parts for every million. The vinyl hydrogen has distinctive substance move esteem. The methyl aggregate contains essential hydrogen with least concoction move an incentive in parts for every million. The epoxide indicates distinctive part per million estimation of synthetic move.

GG-33 (Formononetin)

^{13}C NMR

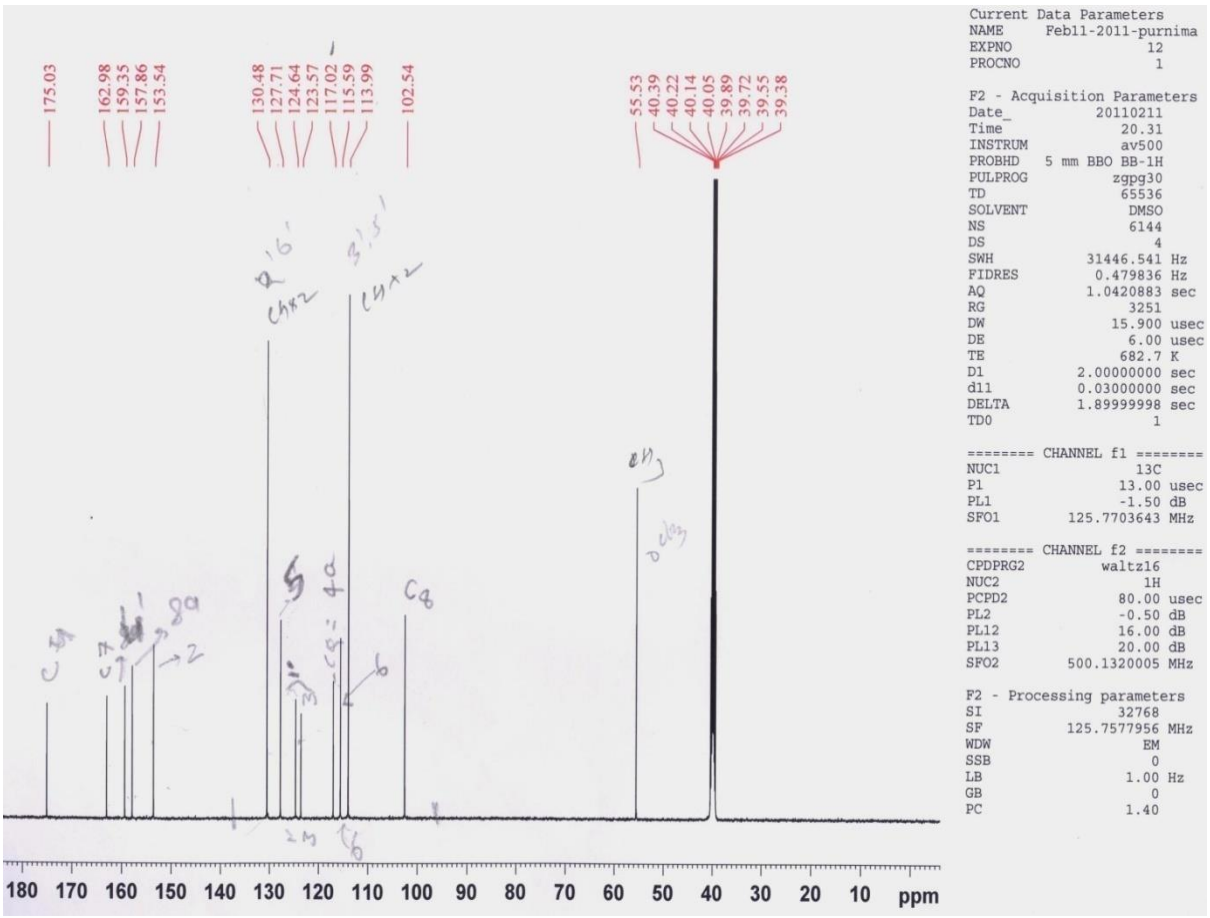


Fig 10: ^{13} C-NMR of Formononetin

Names of Atom	**Chemical shift**	**Interpretation**
Aromatic carbons	26-28.3 p. p. m.	neighboring group effect
Aliphatic carbons	22-23.2 ppm	splitting of signal
Vinyl group carbon (-C)	2 3.5-4 ppm	intensity is fast
Hydroxyl group hydrogen	20.1-22.5 ppm	number of signal
Epoxide carbon	20.3-20.4 ppm	electronic

On the basis of ^{13}C nuclear magnetic Resonance spectra (NMR) the compounds have aromatic and aliphatic carbon that are identified on the basis of chemical shift value in parts per million (delta. The vinyl hydrogen has different chemical shift value. The methyl group contains primary carbon with minimum chemical shift value in parts per million. The epoxide shows different part per million values of chemical shift. The chemical shift value for carbon is twenty times more and the intensity of the compound is four times less as compared to hydrogen nuclear magnetic resonance.

GG-33 (Formononetin)

DEPT (135)

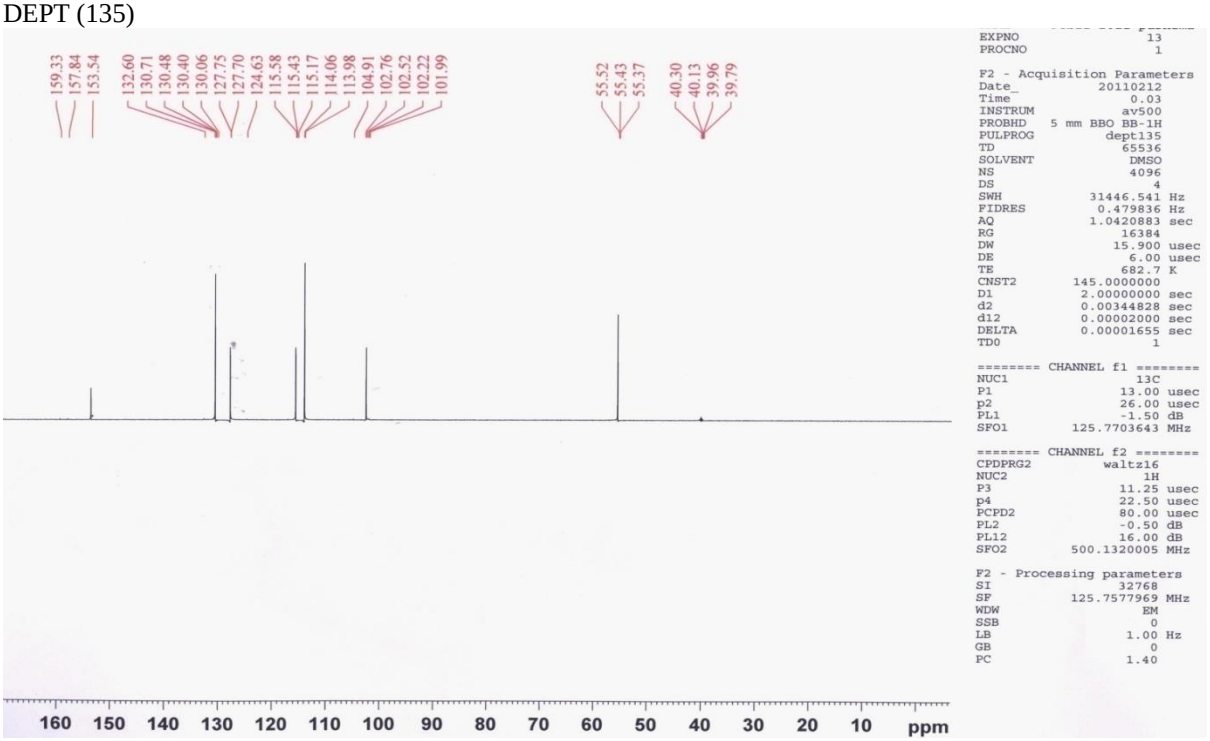


Fig 11: DEPT of Formononetin

Name of atom	**Chemical shift**	**Interpretation**
Aromat. deuterium	7-8.4 ppm	neighbor groups effect
Aliphat. Deuterium	2-3.2 ppm	splitting of signal
Vinyl group deuterium	3.5-4 ppm	intensity is fast
Hydroxyl group deuterium	2.1-2.5 ppm	number of signal
Epoxide deuterium	2.3-2.4 ppm	nil
Methyl group	1.2-1.5 ppm	primarydeuterium

On the premise of deuterium atomic attractive spectra (2D NMR) the compound has sweet-smelling and aliphaticdeuterium that are recognized on the premise of synthetic move an incentive in parts for each million. The vinyldeuterium has diverse synthetic move esteem. The methyl amass contains primarydeuterium with least synthetic move an incentive in parts for every million. The epoxide demonstrates diverse part per million estimation of synthetic move (ppm).

GG-33 (Formononetin)

ES-MS

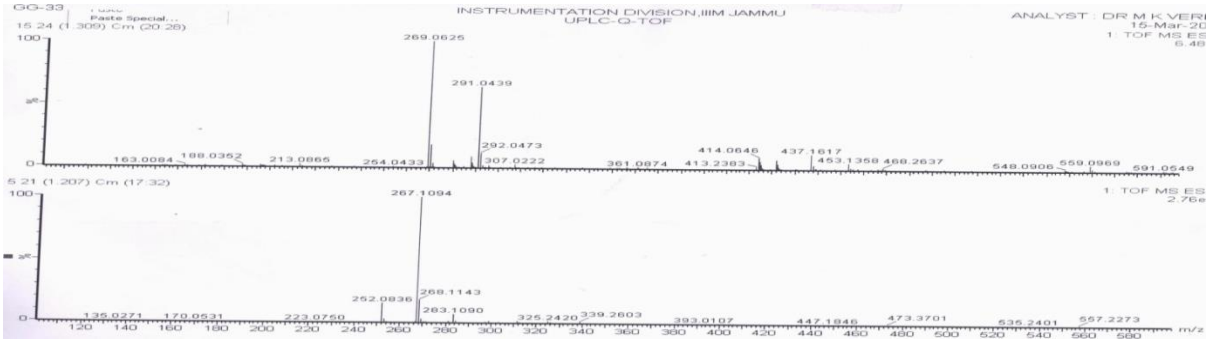


Fig 12: Mass Spectra of Formononetin

According to molecular mass we can determine the molecular weight of the compound. We know that the molecular ion peak present the mass or molecular weight of the uknown compounds. In the given figure the base peak shows the peak at 267.435 that is the mass of the product. The parent peak is known as base or molecular weight of the compound. The fragementations of the compound shows breaking points and metabolites of the compounds that is very helpful for structure elucidation of the compounds. The m+1, m+2, m+3 etc. Peaks are known as isotopic peak of the compound. These pinnacles are exceptionally useful to decide the sub-atomic fornula of the obscure mixes. These two things sub-atomic weight and sub-atomic formular are vital determinant of the auxiliary clarification of the unkonw compound. We speak to relative power or wealth on the y-hub and sub-atomic mass on the x-pivot for translation. These are very useful for structrure elucidation of the unknown compounds.

Biological Assays

Pure CompoundsGG-09, GG-47 isolated from *G. glabra* ethanolic extract was evaluated for their *in vitro* biological activities which include: - Antibacterial, Antifungal activity along with original crude.

Agar-Well Diffution Method for Antimicrobial Screening

Test organisms (Bacteria)

Methicillin Resistant *Staphylococcus aureus* (MRSA)

Vancomycin Resistant *Enterococcus faecalis* (VRE)

Pseudomonas aeruginosa ATCC 27583

Fungal

C&ida albicans (FCZ^r)

Aspergillus fumigatus

Assay Media

Bacteria: Muller Hinton agar (Difco, USA)

Fungal: RPMI supplemented with 0.165M MOPS & 1.5% agar (Sigma)

Standard antimicrobial agents used

Ciprofloxacin - 5µg/50µl

Amphotericin B - 1 µg/50µl

Screening of extracts for antimicrobial activity

1. The inoculums were prepared in sterile normal saline of test organisms from the over night growth on trypticase soya broth (for bacteria) & sabouraud dextrose agar (for fungi). For *Aspergillus fumigatus*, used the conidial suspention.
2. Turbidity was adjusted to 0.5 Mc Farl& (equivalent to 1.5 x 10^8 CFU/ml of *E.coli*& 1 x 10^6 CFU/ml of *C. albicans*) using a densitometer. 3.500µl of McFarl& adjusted cultures was added to 50 ml of sterile molten agar (45 - 50 ^{0}C). Muller Hinton agar was used for bacterial cultural & RPMI media for fungal cultures. It was mixed & poured in to PD150 sterile plastic plates. Allowed it to set & then individual plates were marked with the organism inoculated.
1. punched the wells (6 mm diameter) & 50µl of sample (extract) was added to it. Appropriate standard antibiotic was used as a control.
2. Plates was Incubated at 37^{0}C for 24 to 48hrs.

Zone of inhibition was measured & results were recorded.

Table 1: Antibacterial & Antifungal activities of Compounds

S.No.	Tested extracts	Sterility	*Tested organisms (Zone Diameter (in mm))*				
			Bacterial Pathogens			**Fungal Pathogen**	
			MRSA	**VRE**	***P. aeruginosa***	***C.albicans* (FCZ**	***A. fumigatus***
1.	Crude	NG	0	0	0	0	0
2.	GG-09	NG	4	4	0	2	2
3.	GG – 47	NG	-	-	-	-	-
4.	GG-33	NG	0	0	0	0	0
5.	DMSO control	NG	0	0	0	11^H	0
6.	Drug control	NG	0	0	32	20	21

Here H = Hazy zone of inhibition, G = Growth & NG = No Growth. Ciprofloxacin (5µg/well) & Amphotericin-B (1µg/well) were used as a standard antibacterial & antifungal agent respectively in this study.

RESULTS & DISCUSSION OF ANTIBACTERIAL ACTIVITY

The Ethanol extract of rhizome of *G. glabra* was chromatographed over silica gel column & then final purification achieved by sephadex (LH-20). compounds, Formononetin (GG-33), Hispaglabridin A(GG-09), Glicyrrazic acid were isolated & their structure was determined by spectroscopic methods.. The major compound Glycyrhine was hydrolyzed to get aglycone Glycyrrhetic acid.

Compounds were screened for Antibacterial & Antifungal activity, resulted was tabulated in Table 4. However, the crude was not showing any activity (250µg/ml) but pure compounds have different activity pattern against tested organism. None of the compound was found to be active against *P. aeruginosa*.

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