Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
5(1), 2016, 16-35

ISSN 2321 – 0958

Original Research Article

PHYTOCHEMICAL, CYTOTOXIC INVESTIGATION AND MOLECULAR DOCKING STUDIES OF CYTISUS SCOPARIUS Link FOR ITS DUAL HUMAN TOPO POISONING (I & II) ACTIVITY.

M. V. N. L. Chaitanya[1]*, S. P. Dhanabal[1], S. Jubie[2] & M.Pavithra[1]

1, Department of Pharmacognosy and Phytopharmacy, JSS College of Pharmacy (JSS University), Rockland's, Ooty-643001, Tamilnadu, India.

2. Department of Pharmaceutical Chemistry, JSS College of Pharmacy (JSS University), Rockland's, Ooty-643001, Tamilnadu, India

Highlights:

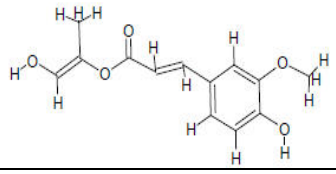
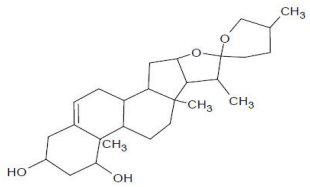
- The aerial parts of weed plant *Cytisus scoparius* (L.)Links have good amounts of phenolics, flavanoids and saponins.
- A novel phenolic compound (1E)-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate (CH2) was isolated and characterized first time from this plant using Flash chromatography.
- First time, the total saponin fraction was isolated and characterized by LCMS finger printing and found to be have ruscogenin as one of the saponin
- The total saponin fractions and isolated compound showed significant antioxidant and anticancer properties.
- To study their mechanism of action for cytotoxicity these molecules have been docked into the crystal structure of Topo –I and Topo-II enzymes usingSchrödinger suite, 2014-3.

Introduction: Generally, weeds are considered as nuisances in the garden and enemies to the farmer, as there is a misconception that they are useless. Many of the herbs used in Indian traditional medicine and tribal medicine are considered as weeds by agriculturists and field botanists (for example, *Phyllanthus amarus* L., *Eclipta alba* L., *Centella asiatica L.*, etc.). Even though many of these weeds have high ethno pharmacological importance, they are being destroyed and there is a lack of scientific knowledge and guidance. In the Nilgiris, many medicinally valuable weeds like *Achyranthes bidentata* Blume., *Artemisia nilagirica* Clarke., *Centella asiatica* L., are very prominent having good therapeutic values like diuretic, antimalarial and brain tonic. Due to a misconception that the weeds are useless; many of these weeds are not explored phytochemically and biologically, there is a good chance for the pharmacognosy scientists

For Correspondence:
chaitanya.phyto@gmail.com
Received on: December 2015
Accepted after revision: March 2016
Downloaded from: www.johronline.com

to do an extensive research on these weeds for the discovery of new lead molecules[1]. In countries like Thailand, India and Brazil, these weeds are using in traditional system of medicine for several diseases like cancer, microbial infections etc., which proved that these weeds also having some potential moieties which are responsible for various biological activities. However these weeds are not explored thoroughly, hence there is a lot of chances for scientists to discover new leads against challenging diseases like cancer[2].

COMPOUND SELECTED FOR THE PRESENT STUDY

S.NO	Name of the compounds (molecular weight)	Structure
1.	**(1E)-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate (CH2) (250.2)**	
2.	**Ruscogenin (430.6)**	

In other words Cancer is a defined as a circumstance which arises while a cell starts disobeying the check mechanisms, which control the rate of cell proliferation and starts di- viding in an uncontrolled manner. This leads to the for- mation of a neoplastic tumor, which is normally benign at this stage, but becomes malignant, when it starts spreading to other tissues. Cancer arises mainly due to two reasons: 1) Gain of function of a proto-oncogene, and it becomes oncogenic; 2) failure of the function of a tumor suppressor gene[3].

The discovery of new leads against cancer is possible only through natural drug discovery process. Even though, drug discovery from nature is a tedious process, every first lead molecule like aspirin is a result of nature only. So, the natural drug discovery process is in demand but still with technical advancements like high through put screening, flash chromatographic techniques and *in silico* studies, the natural product drug discovery became easier and fast, Hence there is a high demand for these techniques over all the globe[4].

With the invention of virtual screening (VS) techniques, the drug discovery process became easy, within the last five years there is a high demand for these types of publications having various new computational drug discovery tools. Many of these methods are employed to identify potential ligands for protein targets of pharmacological/ therapeutic interest. These *in silico* tools are best applied in natural medicine research as means to identify the potential mechanisms of the isolated molecules and their targets. As there is a high cost involvement in using in *vitro* and *in vivo* techniques, *in silico* tools offers an economical and efficient way of exploring the different molecular mechanism of the lead molecules against various ailments like cancer, tuberculosis, microbial infections etc[5].

According to the current research, the main targets in treatment of cancer are Human topoisomerases I & II, therefore all the scientists are in search of new leads which having dual human top poison I & II activity. So there is a much demand for these types of natural leads in the research market[6-8].

Cytisus scoparius (L.)Link, also known as scotch broom, belongs to family fabaceae is a deciduous Shrub growing to 2.4 m (7ft) by 1 m (3ft 3in) at a fast rate. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Bees. The plant is not self-fertile. It can fix Nitrogen and it is noted for attracting wildlife. Broom is a bitter narcotic herb that depresses the respiration and regulates heart action. It acts upon the electrical conductivity of the heart, slowing and regulating the transmission of the impulses. The young herbaceous tips of flowering shoots are cardio tonic, cathartic, diuretic, emetic and vasoconstrictor. The plant is used internally in the treatment of heart complaints, and is especially used in conjunction with *Convallaria majalis*. The plant is also strongly diuretic, stimulating urine production and thus countering fluid retention.

However the phytochemical study on this plant has not carried out in-depth and no studies was carried out on this plant as a cytotoxic agent. Keeping all these facts as a positive aid for the current research. The present work is focused to investigate the phytochemical and cytotoxic nature of c*ytisus scoparius* for the discovery of new human dual topo I & II poisons as a lead molecules against cancer.

Materials &Methods

Microscopical Authentication: The plant material (Whole plant) used in the present study was collected from Udhagamandalam in June 2013, The Niligiris district, Tamilnadu, India and was authenticated by the field taxonomist Dr. S. Rajan, Survey of Medicinal Plants and collection unit, Department of Ayush, Emarald and the voucher specimen was deposited at Department of Pharmacognosy and Phytopharmacy, JSS College of Pharmacy, Ooty (JSS University, Mysore) with identification no (JSSCP.PhD001/14). The Microscopical studies of the collected fresh material (leaf and stem) were carried out at Plant Anatomy Research Centre, Chennai, Tamilnadu, India. Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations of bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against a dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books [8 – 10].

Extract Preparation : The whole plant of Cytisus scoparius (L.)Link, was collected and shade dried for 7 days. 1kg of dried, finely powdered material was triple macerated using 3 L of 70% v/v hydro-ethanol for 72 hrs. The obtained filtrate was subjected to vacuum distillation using Buchi Rotavap (R-210). The obtained extract was dried in a desiccator and the percentage yield was calculated on dried basis[11-12.]

Fractions Preparation: 20g of above obtained extract was dissolved in 50 ml of double distilled water and subjected to liquid-liquid fractionation using different solvents like 200 ml of n-hexane, 500 ml of chloroform, and 500 ml of ethyl acetate. The obtained filtrate was subjected to vacuum distillation using Buchi Rotavap (R-210) and the percentage yield of obtaining fractions was calculated on dried basis[13 – 14].

Phytochemical Screening

Qualitative Phytochemical Screening: The prepared extract and fractions were subjected to a preliminary qualitative phytochemical screening following the methods based on standard protocols [15].

Quantitative Phytochemical Screening: The prepared extract was subjected to total alkaloid, total flavanoid, total phenolic and total saponins estimation based on standard procedures.

Total Alkaloid Content Estimation: The obtained extract was subjected to total alkaloid

content estimation using bromo-cresol green colorimetric analysis [16].

Total Flavanoid Content Estimation: The extract obtained was subjected to total flavanoid estimation using aluminum chloride calorimetric analysis[17].

Total Non-Flavanoid Phenol Content Estimation: The extract obtained was subjected to total phenol content estimation using Folin-Ciocalteu spectrophotometric method [18].

Total Saponin Content Estimation: The total saponin content was isolated from the 100g of crude drug using the gravimetric separation technique. Take 100g sample crude drug powder with 90% v/v methanol (500ml) by refluxing for half an hour. Extract the residue two more times by taking 500 ml methanol. Combine the methanol extract and distill off the solvent. Treat soft extract left after distillation of alcohol, with petroleum ether 60-800c, 500 ml by refluxing for half an hour. Cool and remove the solvent by decantation. Now treat the same soft extract successively with chloroform 500 ml and ethyl acetate 500 ml and pour the solvents after cooling, keeping the soft extract in the same flask.Dissolve the soft extract (after three extractions cited above) in 500 ml of butanol. Filter and concentrate to 100 ml. Add the above drop by drop with constant stirring to 500 ml with Acetone in order to precipitate the saponins. The precipitates are filtered, collected and dried to a constant weight at 105ºc [19].

HPTLC Finger Printing of Total Saponin Fraction: 1g of the isolated fraction was dissolved in 5 ml of methanol and evaporated to about 1ml, mixed with 0.5 ml water and then extracted with 3 ml of n-Butanol. The n-Butanol layer was collected and evaporated used for TLC analysis by using 20 µl as sample was applied on aluminum precoated silica gel 60 GF 254 TLC plate using Chloroform : Methanol : Water (65 : 35 : 10) and the eluted saponin spots were identified under visible light & 366 nm in a TLC Visualiser (CAMAG)[20].

Isolation of Compound (CH2) from *Cytisus scoparius* Link using flash chromatography system: Isolera flash chromatography system having a touch screen display, which is a solvent-resistant, color LCD screen with a resolution of 800 x 600 pixels. It serves both as a display and as the system's input device via on-screen touch controls. A fraction collector, which collects fractions into a wide variety of collection racks and vessels. A pump module, which directs the liquid flow through the system. A default flow rate is specified for each cartridge but, if desired, the flow rate can be changed. If the flow rate is increased, the system will start the run at the default flow rate and then regulate towards the flow rate defined in the method. Note that the system regulates on both flow rate and pressure. If 90% of the maximum allowed pressure is reached before the defined flow rate, the flow rate at 90% pressure will be used. An internal detector, which provides the system with information on the light absorbance of the solvents and samples passing through the detector flow cell. The different fractions can be collected through an automated fraction collector based on the Rf the compound of interest can be identified and pooled together after performing thin layer chromatographic analysis.

5 gms of the 70% hydro-ethanol extract was dissolved in 20 mL of water and sonicated for 25 minutes. The obtained mixture was filtered under vacuum and 10mL of filtrate was partitioned with 60 ml of ethyl acetate (3 x 20 mL). The Ethyl fractions were pooled together and evaporated under Buchi rotavap (R210) leaving a brownish mass. 500mg of the dried powder m was directly applied on 10 gm samplet and samplet was dried under vacuum in rotary evaporator (Buchi R 120).The dried samplet was packed in 10 g KPSil Biotage SNAP Cartridge filled tightly or compactly with 10 gm of Sephadex gel (LH-20). A gradient flash chromatography method was developed based on blank method. A constant flow rate 50

ml/min of mobile phase (Water: Methanol) is used.

***In-silico* molecular docking studies against Topo I & Topo II enzymes:** The molecular docking studies were carried out using the X-ray crystal structures of Human topo isomerases I (PDB ID: 1K4T at 2.10 A˚, PDB ID: 3AL2 at 2.0 A0) and Human topo isomerases II (PDB ID: 1ZXN at 2.51 A˚, PDB ID : 3QX3 at 2.16 A˚) . The docking studies were carried out between the selected isomerase proteins and energetically minimized chemical compound (CH2) using GLIDE Program (Grid Based Ligand Docking from Energetics, from Schrodinger 9.9/2014-3 suite). The Epik studies were carried out for the selected compounds using the same software in order to know their pKa values. The ADMET studies were carried out using QikProp43-44. The binding free energy of inhibitors in the catalytic domain enzyme (1K4T) was calculated by Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) VSGB 2.0 method. The calculated inhibitor-enzyme complex was relaxed using the local optimization feature in Prime, version 4.1, Schrödinger 2014, and the energies of the system were calculated using the VSGB 2.0 (solvation model) method available in Schrödinger suite 2014 (Schrodinger 9.9/2014-3 suite) [21 – 24].

***In-vitro* Antioxidant assay using DPPH and ABTS assay :**

DPPH (1,1diphenyl-2-picryl hydrazyl) radical scavenging activity: 1 ml of test solution added to 1 ml of DPPH in methanol (0.33%). After keeping for 30 minutes at 37˚C the absorbance at 518nm was measured using UV spectrophotometer. Corresponding blanks were taken. The absorbance of DPPH as control was measured at 518 nm. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH accepts an electron to become a stable diamagnetic molecule. The methanolic solution of DPPH (violet colour) has got a strong UV absorbance at 518nm. The presence of a reducing agent in this methanolic solution pairs the odd electrons of DPPH radical and further the solution losses colour stoichiometrically and also the absorbance of the solution decreases at 518nm [25].

The scavenging effect (%) was measured using the following formula:

$$\text{ScavangingEffect(\%)} = \frac{\text{(Control absorbance – Test absorbance)}}{\text{Control Absorbance}} \times 100$$

$ABTS^{\cdot+}$ Assay: The ABTS.+ radical cation decolorization assay was performed to evaluate the radical scavenging ability of test drugs by the standard method50 – 51. ABTS radical cation (ABTS. +) was generated by adding 2.45 mM potassium persulfate to 7 mM ABTS and incubated in darkness at room temperature for 12–16 h. This stock solution of ABTS.+ was diluted with ethanol to give an absorbance of 0.70 (± 0.02) at 734 nm, which act as a positive control. 10 µl of test drug (prepared in ethanol/DMSO) will be mixed with 1.0 mL of diluted ABTS.+ solution and incubated at 30ºC for 30 Min. The absorbance value was measured at 734nm with UV–visible spectrophotometer. Trolox standard will also be prepared (in ethanol: 0–1.5 mM) to get the concentration response curve. The unit of trolox equivalent antioxidant activity (TEA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as mmol/g of extracts[25].

***In vitro* Cyto-toxic Studies Using Sulphorhodamine B (SRB) assay**

Cell Lines and Culture Medium: The cell cultures used in this research were procured from National Center for Cell Sciences, Pune, India. The cells were grown in Earls Minimal Essential Medium supplemented with 2 mmol L-glutamine, 10 % Fetal Bovine Serum, Pencillin (100 µg/ml) and amphotericin B (5µg/ml) and the cells were maintained at 370C in a humidified atmosphere with 5 % CO2 and subculture twice a week.

***In-vitro* Topo Enzyme poisoning Effect**

In vitro cyto-toxic studies were carried on the isolated compounds and fractions based up on the standard protocol and compared with standard quercetin [26].

Results & Discussion

Microscopical Authentication: The leaf is slightly curved on the adaxial side; it has less prominent midrib and thin dorsiventral lamina. Mid rib is plano convex with flat adaxial side and short and broad conical adaxial part. The mid rib is 300 µ m wide. The palaside layer is adaxially transcurrent and extends in between the adaxial epidermis and the vascular bundle. The vascular strand is single, circular and 170 µm in diameter. It includes about four short parallel xylem lines and equal number of small units of phloem. The xylem and phloem are collateral. The vascular bundle is surrounded by a single layer of large circular parenchyma cells. Short wide two celled glandular trichomes are sporadically seen on the abaxial epidermis and are 30 µm in height.

The stem is five winged and appear star shaped in sectional view. It is 1.4 mm thick. The wings are 300 x 400 µm thick. The epidermal layer of the stem is thin and the cells are small and square shaped. The cuticle is very thick , the cortex is parenchymatous, the cells are varied in shape and size; these are small masses gelatinous fibres which occur within the wing and in radial row in the wing. Secondary phloem is in thin continous cylinder encircling the xylem cylinder. The phloem elements are small and compact. Secondary xylem is thick and solid hollow cylinder. It includes small clusters of vessels in the outer part of the xylem cylinder. It includes small clusters of vessels in the outer part of the xylem cylinder. The pith is fairly wide and parenchymatous discussed below in the figure 1.

Qualitative and Quantitative Phytochemical Analysis: The extracts and the fractions were subjected to phytochemical screening and found to contain good content of alkaloids, cardiac glycosides, flavanoids, triterpenoids/steroids, saponins, phenolics, tannins, saponins, carbohydrates, cardiac gylcosides, saponins and coumarins and discussed below in the Table 1 & Table 2.

HPTLC finger printing analysis of total saponin fraction: The saponins with different R_F were identified using high performance thin layer chromatography analysis and the R_F of the prominent spots was discussed below in Table 3 and in Figure 2.

LC-MS Finger printing of total saponin fractions

The isolated total saponin fraction was subjected to LC-MS analysis and a total spectrum was run in both positive mode and negative mode. The sapogenin ruscogenin was identified in positive mode total scan as M+1 peak at 431 along with several unknown saponins (Table 4 and Figure 3).

Flash Chromatographic separation and characterization of isolated compound

A total no of buff coloured 14 fractions, each 18 ml was collected in different test tubes at the wave length of 254 & 366 nm. Each individual fraction was subjected to TLC Analysis and all the 14 fractions were pooled together and evaporated under vacuum using Buchi Rotavap (R 210) in order to give a light creamy mass. The creamy mass is acidified with 1 N sulphuric acid and dissolved in 2 mL of hot water and on evaporation leaving white crystals of 30mg of compound (CH2). The isolated compounds were identified by phytochemical test, melting point, TLC analysis and structural elucidation were characterized by using IR, NMR & LC-MS Spectral studies (Fig 4, 5, 6, 7 & 8) & (Table 5).

***In-silico* molecular docking studies:** *In-silico* studies were carried in order to know the mechanism of action of the isolated compounds(CH2 and ruscogenin) as anticancer agents in comparison to standard quericitin. All these compounds showed good g-scores against topo enzymes I & II. All these compounds are having good binding capacity and good ADMET profile. The isolated compounds

(CH2) and ruscogenin showed and the results were discussed in the below table 6.

***In-vitro* antioxidant and cytotoxic studies:** In-vitro antioxidant studies using DPPH and SRB (Sulphorhodamine B) assays, In-vitro cytotoxic studies on Vero, A-549, MCF-7 & HCT-116 using SRB (Sulphorhodamine B) method were carried out using extracts, different fractions & isolated compound in comparison to standard quericitin. The total saponin fraction and isolated compound (CH-2) showed good antioxidant and anticancer activities (Table 6). The cytotoxic activity of these compounds is may be due to inhibition of human topo isomerase enzymes I & II which is proved by molecular docking studies as discussed in Table 7.

Discussion :

The selected plant *Cytisus scoparius* Link is predominantly distributed and less explored weed of the Niligiris region. The selected plant has richest sources of phenolics, saponins , glycosides & Alkaloids. Hence the plant is selected for the phytochemical isolation of novel anticancer leads. The total saponin fraction which is isolated from this plant possess good antioxidant and cytotoxic activity. Based on the chemotaxonmic significance of saponins. The total saponin fraction isolated was subjected to LC-MS finger printing analysis. Based on the mass profiling, it is found that the total saponin fractions contains many important saponins, out of which a well known sapogenin ruscogenin was traced out along with other saponins .

First time a novel phenolic compound (CH2) was isolated from this plant by using a flash chromatography technique which is a simple, economical and fastest method. The developed method can be used by natural product scientists for the isolation of saponins. Based on the docking scores, the isolated compounds (CH2) and ruscogenin proved that these are a very good dual human topo poisons I & II which is an essential criteria on which the current anticancer drug discovery is dependent on. The ADMET studies proved that these compounds are safe and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) studies prove that these molecules having good binding surface areas against target enzyme.

Conclusion

Based on the biological screening and molecular docking studies, the isolated compound (CH2) proved that it is a good cytotoxic molecule and the mechanism of apoptosis is due to dual human topopoisoning I & II activity. As there is a current demand for these novel dual human topo poisons, the selected weed may be have a richest source of many secondary metabolites which can be a good lead molecules for discovery of dual human topo poisons I & II. The isolated compounds (CH2) and ruscogenin can be a good lead molecules in anticancer drug discovery. However, further in-depth studies have to be carried out on these identified molecules and the plant in order to prove its clinical significance as good anticancer drugs and the work is in progress in our research laboratory. The isolated molecules can serve as lead molecules towards anticancer drug discovery. The current research work may give a platform for the discovery of novel dual human topo I & II isomerase poisons, where there is a current demand in the anticancer research.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contribution

Mr. MVNLC (M. V. N. L. Chaitanya) carried out all the studies, analyzed the data and drafted the manuscript. Dr. SPD guided the entire research work with his valuable ideas and suggestions. Dr SJ (S. Jubie) helped at Phytochemical work, compiling and editing the final draft. Mrs. MP (Motamarri Pavithra) helped in carrying out the biological studies, All authors have read and approved the final manuscript**.**

Acknowledgements

The authors greatly acknowledge for financial grant under the Department of Science and Technology (DST) (SR/SO/HS-0288/2012) Government of India for purchasing the flash chromatography instrument (ISOLERA, Biotage), The authors also acknowledges the SRM University , Chennai , Tamilnadu, India for helping in Spectral analysis & JSS University, Mysore, India and JSS College of Pharmacy, Ooty, Tamilnadu for providing the necessary facilities and encouraging our team to carry out the research.

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Table 1 Qualitative phytochemical analysis of *Cytisus scoparius* Link extracts and fractions

S.NO	**Name of the test**	***Erigeron karvinkianus***				
		ME	**PF**	**CLF**	**ETF**	**AQF**
1.	Test for Alkaloids	++	+	++	+	+
2.	Test for Cardiac glycosides	++	-	-	+	++
3.	Test for Flavanoids	++	-	-	++	++
4.	Test for Triterpenoids/ Steroids	++	+	++	+	+
5.	Test for Saponins	++	-	-	-	++
6.	Test for Napthoquinones	-	-	-	-	-
7.	Test for Phenolics	++	-	-	+	++

8.	Test for Carbohydrates	++	-	-	+	++
9.	Test for Coumarins	++	-	-	+	++
10.	Test for Tannins	++	-	-	-	++

(++ - Abundance, + - present and - -ve) (ME – 70% Hydro-ethanol mother extract, PF – Pet-ether fraction, CLF – Chloroform fraction, ETF – Ethyl acetate fraction, AQF – Aqueous fraction)

Table 2 Quantitative Phytochemical Analysis of Cytisus *scoparius* Link hydro-ethanol extract

Total alkaloid Content	**Total flavanoid Content**	**Total phenol Content**	**Total saponin Content**
106.27 µg/g atropine equivalent	81.85 µg/g quercetin equivalent	91.94 µg/g gallic acid Equivalent	8.05 % w/w

Table 3 TLC Finger printing analysis of saponins in total saponin fraction

S.NO	**Name of the Fractions**	**R_F of saponin spots**
1.	Total saponin fraction	0.24, 0.25, 0.45, 0.66 & 0.68

Table 4 LCMS identification of saponins in total saponin fraction

S.NO	**Identified Saponin**	**Molecular Mass**	**Mass peak**
1.	RUSCOGENIN	430	431 (M+1) Peak in positive mode scan.

Table 5 Characterization of isolated molecules

Compound name & Physical state	**Phytochemic Test**	**Melting Range**	**R_F (cm)**	**Characterization**			
				IR	**^{1}H NMR**	**^{13}C NMR**	**LC-MS**
Compound (CH2) (6%w/w, White fine powder)	Ferric chlorid test	173 to 176 ºc	 0.62 at uv 254nm	608 (Ar C-H) 3374 (O-H Str) 1629.38(C=O Str) 1404 (C-H) CH3 Str	□7.1(s) (CH) □6.22 (s)(CH) □3.84 (s)(CH3) □6.19 (s)(CH) □1.96(s) (CH3	□116.18(C) □128.7(CH2) □156(C)	(M+1) Peak at +ve mode 251.09

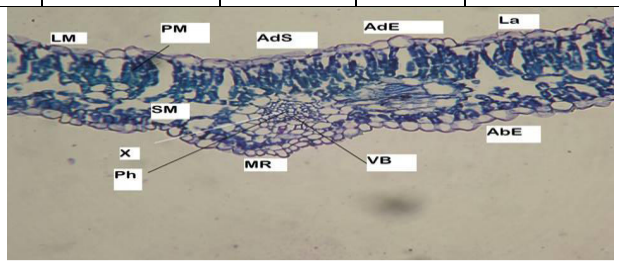
Table 6 G-Scores from the glide XP docking run of standards& isolated compounds with 1K4T & 3AL2 (TOP-1), 1ZXN & 3QX3 (TOP-II)

S.NO	Name of the Compound	Topo-I enzyme Targets							
		1K4T				3AL2			
		G-Scor	H-bond	Penalties	Epik-stael	G-score	H-bond	Penalties	Epik-stael
1.	Std quercetin	-6.7	-2.65	0	0.01	-4.95	-2.44	0	0.01
2.	Std Camptothecin	-4.49	-1.29	1	0	-3.84	-5.14	0	0
3.	Ruscogenin	-3.86	-1.1	0	0	-2.71	-2.17	1	0
4.	CH2	-6.14	-2.44	0	0	-4.02	-2.62	0	0.01
		Topo- II enzyme Targets							
		1ZXN				3QX3			
S.NO	Name of the Compound	G-Scor	H-bond	Penalties	Epik-stael	G-Score	H-bond	Penalties	Epik-stael
1.	Std querceitin	-9.57	-4.65	0	0.01	-7.24	-2.29	0	0.01
2.	Std Salvicine	-7.05	-2.31	0	0	-6.90	-0.48	0	0
3.	Ruscogenin	-4.74	-0.66	0	0	-	-	-	-
4.	CH2	-7.53	-3.11	0	0	-6.59	-0.48	0	0
			Quick PROP 3.4 predictions of ADMET for the compounds						
S.NO	Name of the Compound	CNS	Mol wt	Donor HB	Acceptor HB	QPlog HERG	Human oral Absorption	Rule of 5	Rule of 3
1.	Std quercetin	-2	302	4	5.25	-4.96	2	0	1
2.	Std Salvicine	-2	330	2	6.45	-4.80	3	0	0
3.	Ruscogenin	0	430	2	4.9	-4.15	1	0	1
4.	CH2	-2	250	1	5.5	-4.81	3	0	0
S.NO	Name of the Compound	Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) with 1K4T (Topo-I) Enzyme Target							
1.	Std camptothecin	-91.5039							
2.	Std quercetin	-79.6387							
3.	Std Salvicine	-98.8745							
4.	Ruscogenin	-97.3967							
5.	CH2	-41.1026							

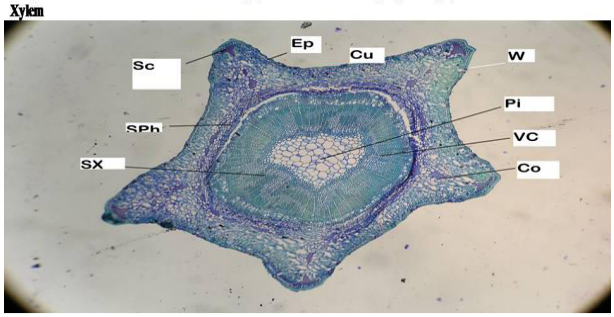
CNS: predicted central nervous system activity on a -2 (inactive) to +2 (active) scales, Mol. wt: Molecular weight of the molecule, Donor HB: Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution, AcceptHB: Estimated number of hydrogen bonds that would be accepted by the solute to water molecules in an aqueous solution, QP Log Her G- Rule of 5: Number of violations of Lipinski's rule of five & Rule of 3 Number of violations of Jorgensen's's rule of three

Table 7 In-vitro Antioxidant & Cytotoxic Screening of Total Saponin Fractions & Isolated compounds

S.No	Name of the Compound	Antioxidant assay IC_{50} Values (µg/ ml)		SRB assay CTC_{50} Values (µg/ ml ± SEM)				
				Cell lines				
		DPPH	ABTS	Concentration (µg/ml)	Vero	A-549	MCF-7	HCT-116
1.	(Total saponin fraction)	272.1	55.4	1000 500 250 125 62.5	440.08±2.9	295.833±1.3	96.333±1.4	206.917±3.9
2.	CH2	25.2	18.3	1000 500 250 125 62.5	383.833±1.8	74.083±1.5	66.5±1.0	50.583±1.3
3.	Std Quericitin	6.94	7.9	1000 500 250 125 62.5	390.6±0.5	155.3±0	36.25±0.1	20.0± 0.1



AbE – Abaxial Epidermis, Ads- Adaxial side, AdE- Adaxial epidermis, BS – Bundle sheath, La- Lamina, LM- Leaf Margin, MR- Mid rib, PM – Palaside Mesophyll, Ph- Phloem, Sm- Spongy Mesophyll, VB – Vascular bundle, X- Xylem



Co - cortex, Cu – Cuticle, Ep- Epidermis, Sc- Sclerenchyma, SPh – Secondary Phloem, SX- Secondary Xylem, Ve – Vessel, XF – Xylem fiber, VC – Vascular cylinder, W – Wing,

Figure 1. Transverse Section of *Cytisus Scoparius* Link aerial parts (leaf and stem)

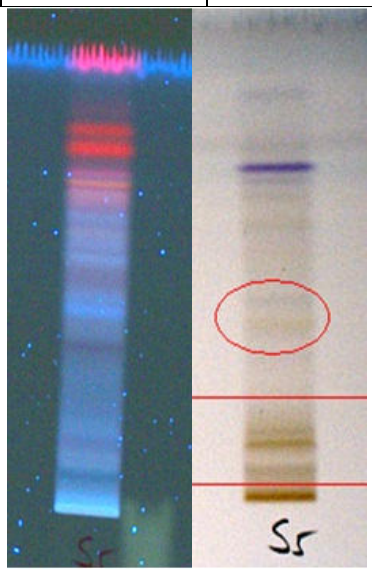


Figure 2. HPTLC Finger Printing of Total Saponin Fraction at 366 nm and under visible light after derivatized with 1% vanillin sulphuric acid reagent

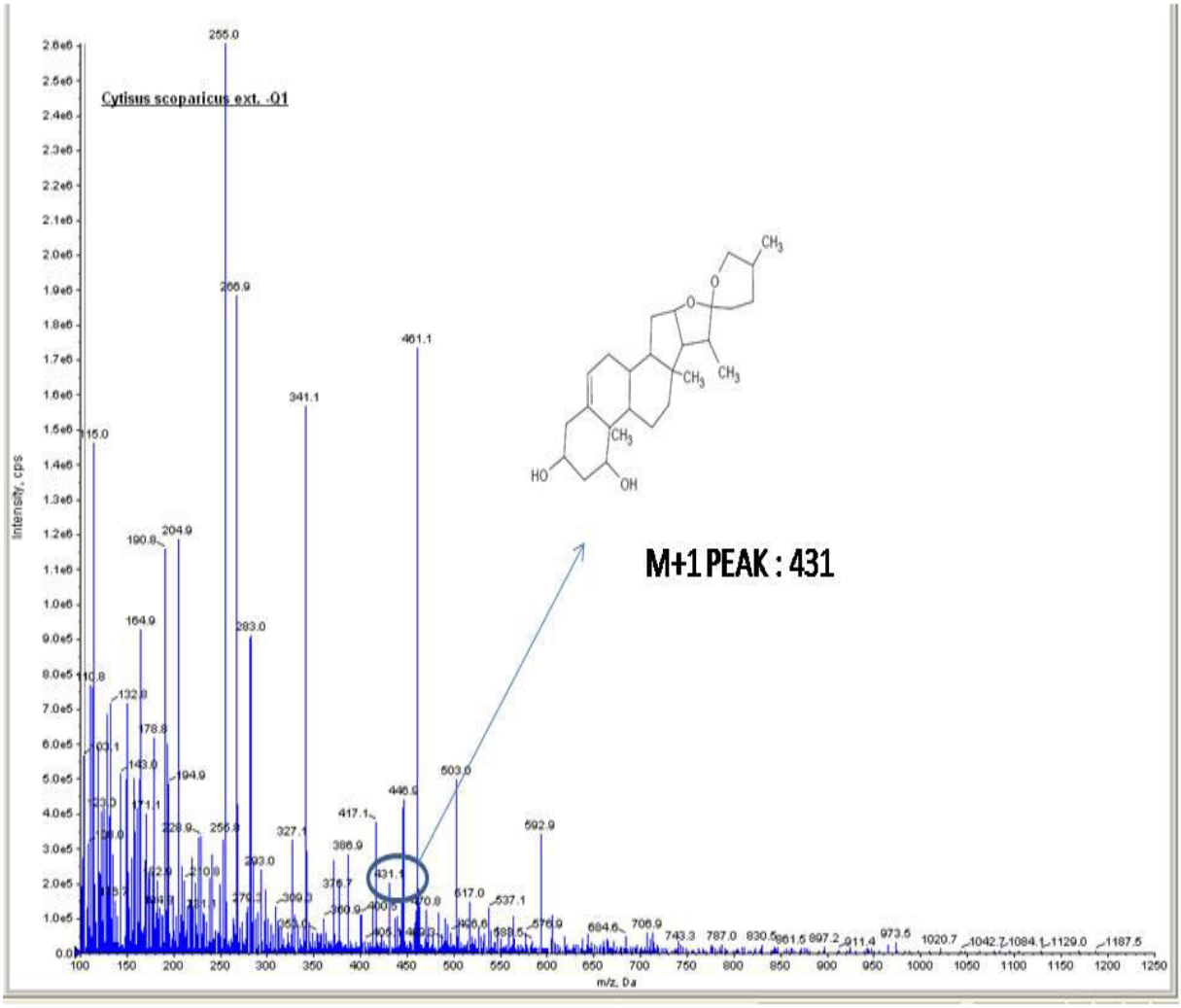


Figure 3. LC-MS ((LC/ESI-MS – ve Mode) Fingerprinting of *Cytisus Scoparius* Link total saponin fraction

Biotage

Isolera™ Fractions Report cs with reversephase (sephadex LH20) 1.

User	chaits	**Cartridge**	SNAP 10g
Sample Name	cs with reversephase	**Rack Type**	16x150 mm
Date	2014-Oct-08 14.43	**Max Fraction Volume**	18 ml
Method		**Solvent A**	Water
Detection Mode	UV1	**Solvent B**	Methanol
UV1 (Collection)	366 nm		
UV2 (Monitor)	254 nm		

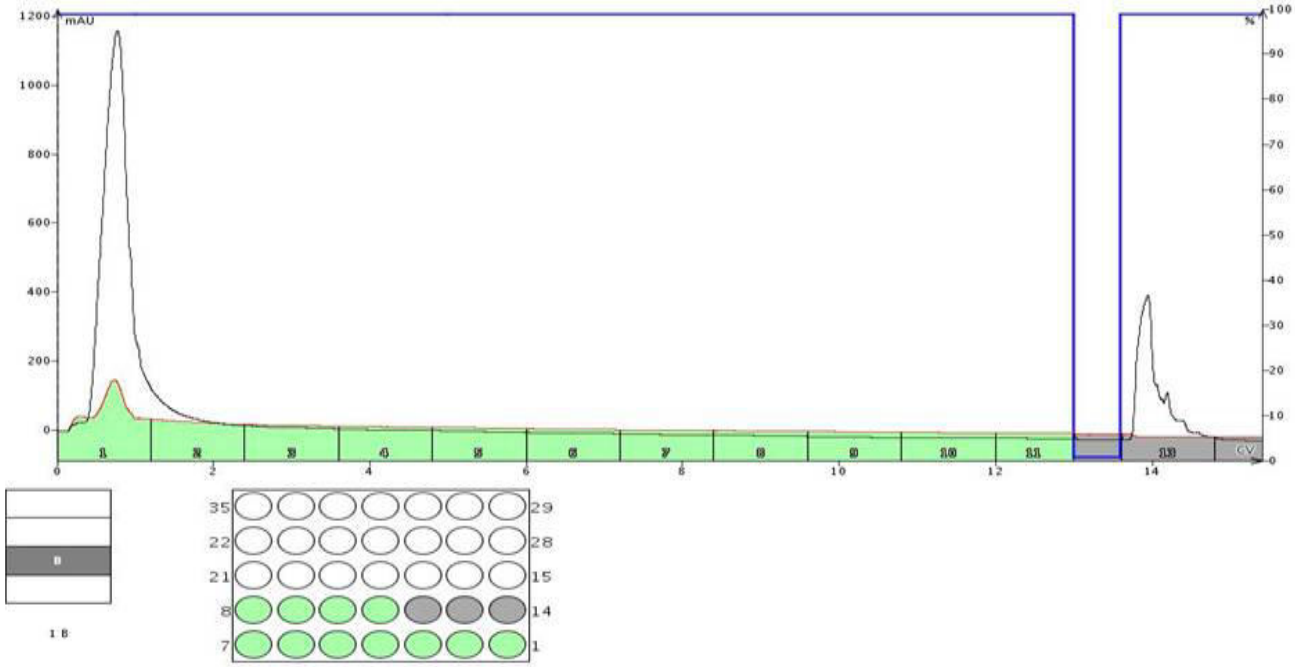


Figure 4 Flash chromatogram report of *Cytisus Scoparius* Link total saponin fraction

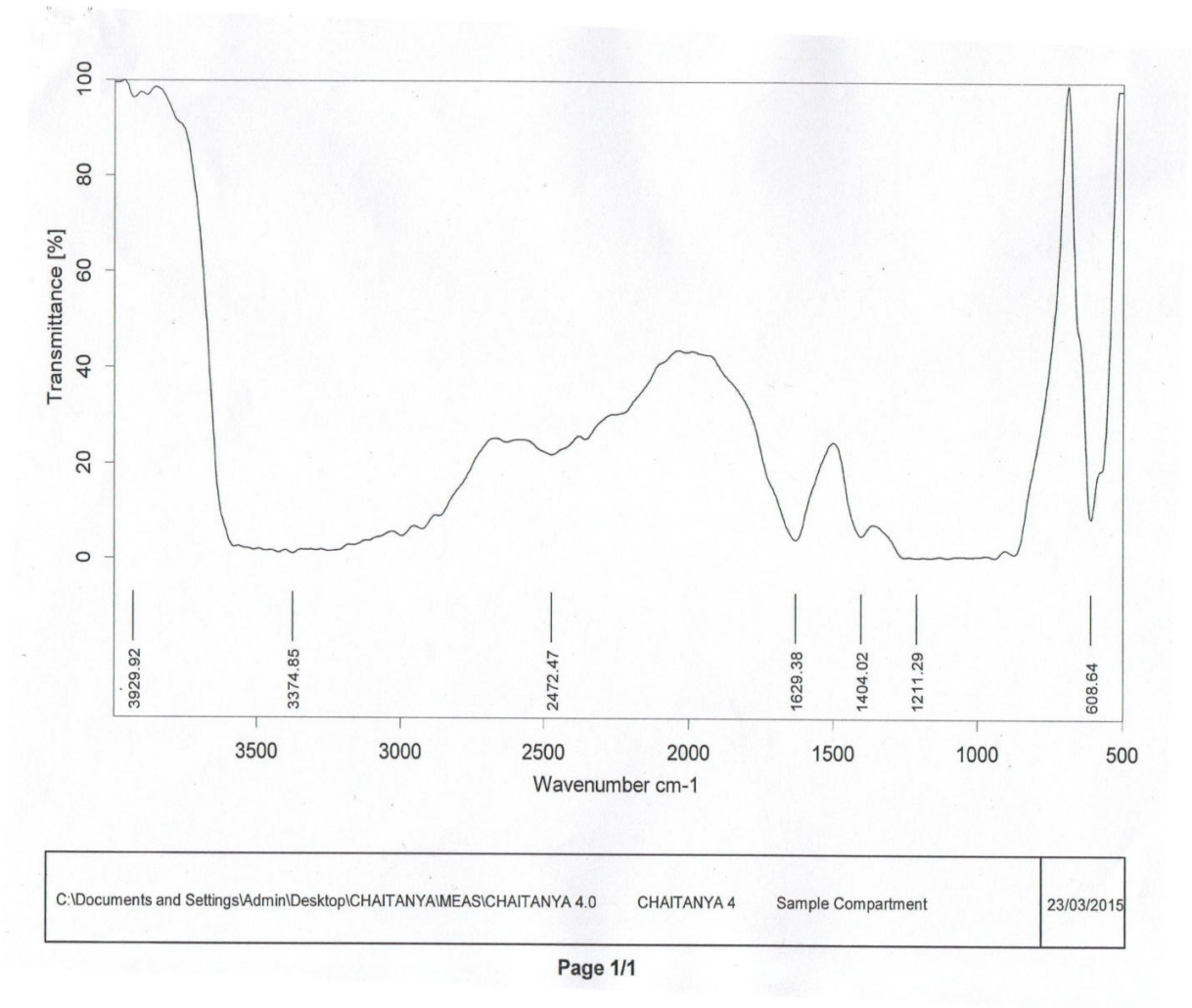


Figure 5 IR spectra of isolated compound (CH2)

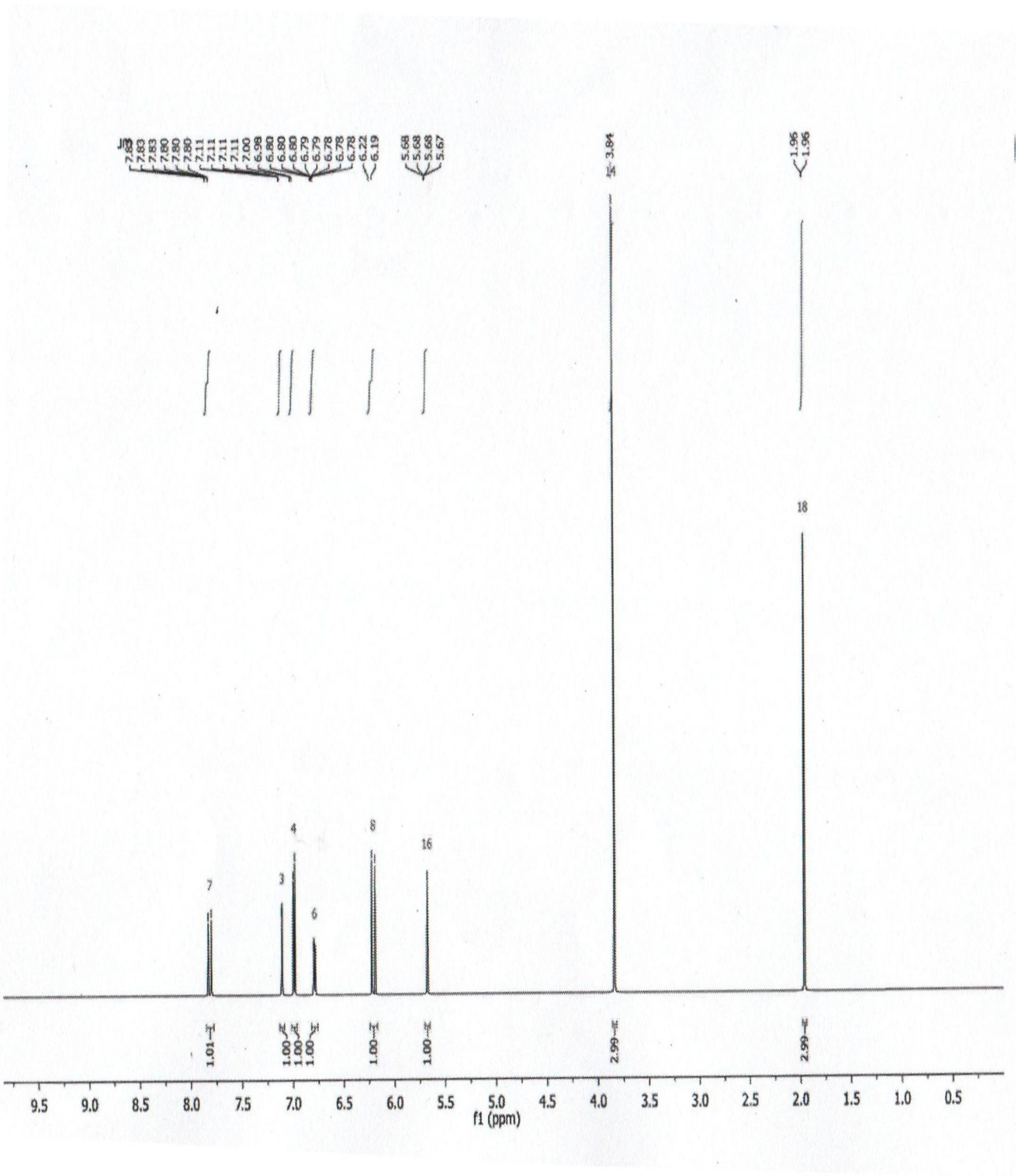


Figure 6 [1] H- NMR spectra of isolated compound (CH2)

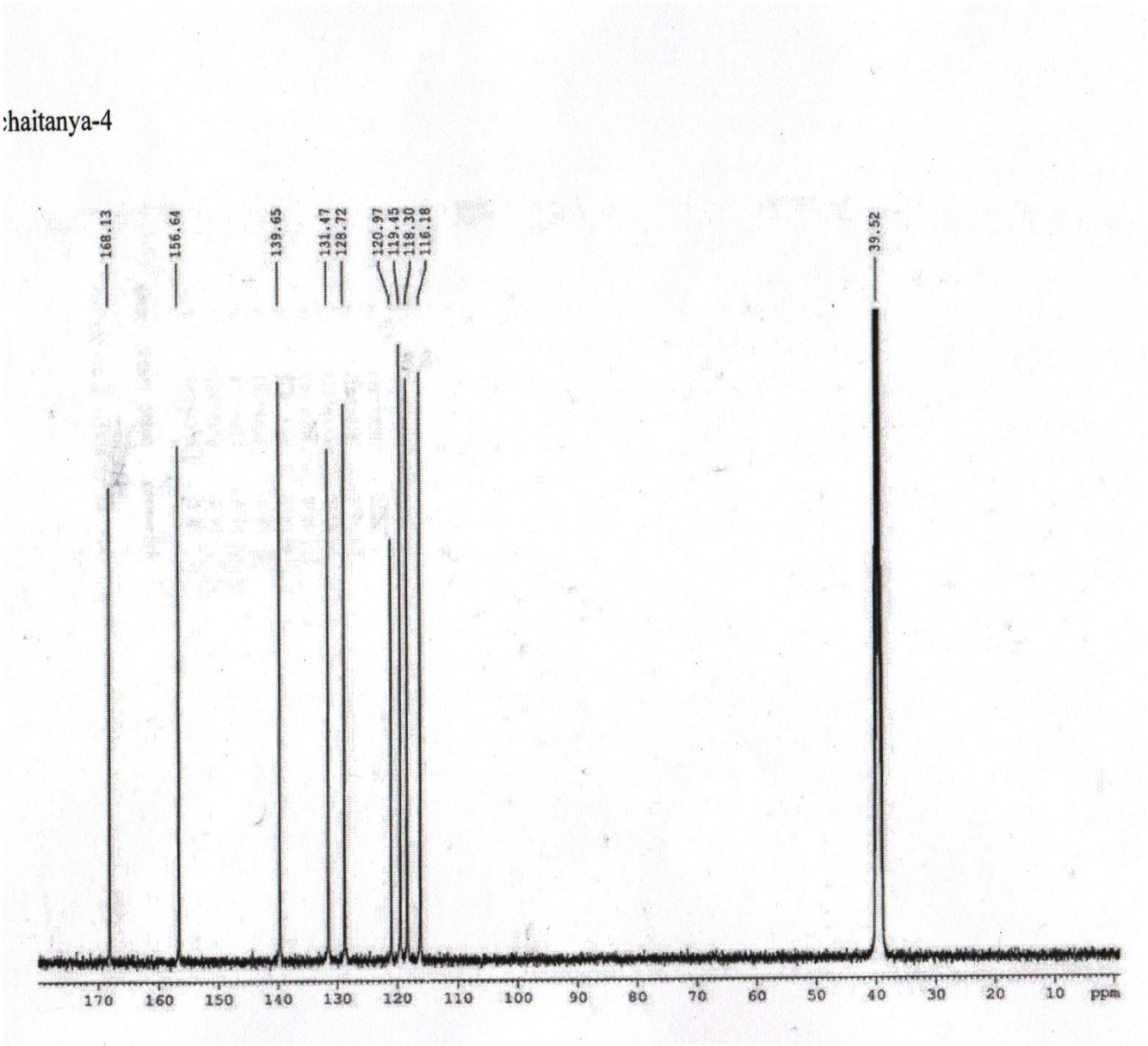


Figure 7 ^{13}C- NMR spectra of isolated compound (CH2)

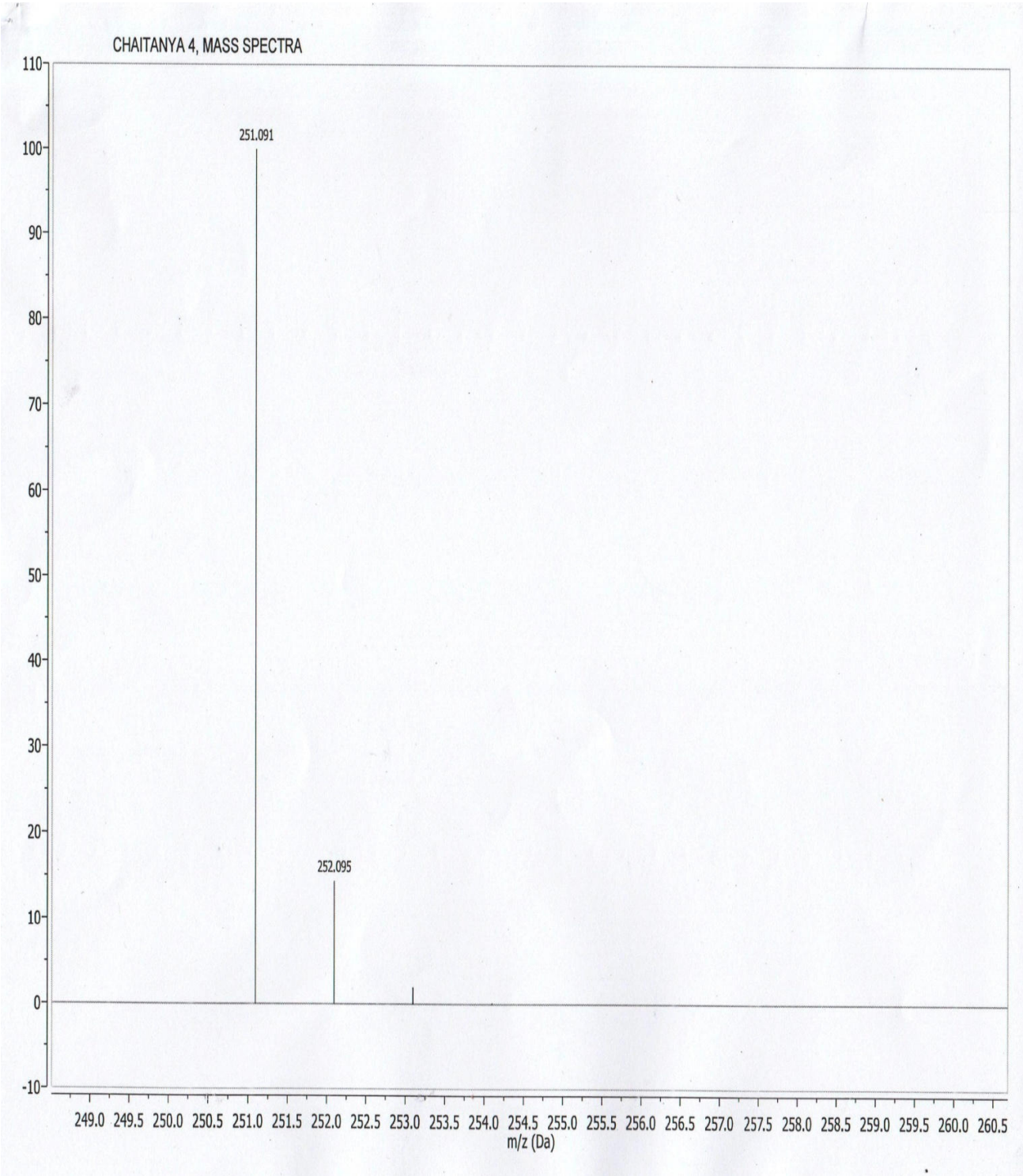
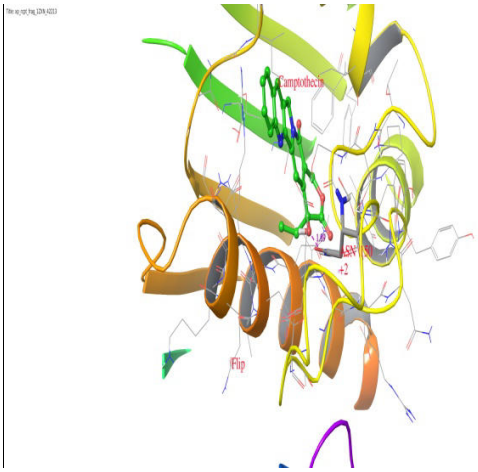
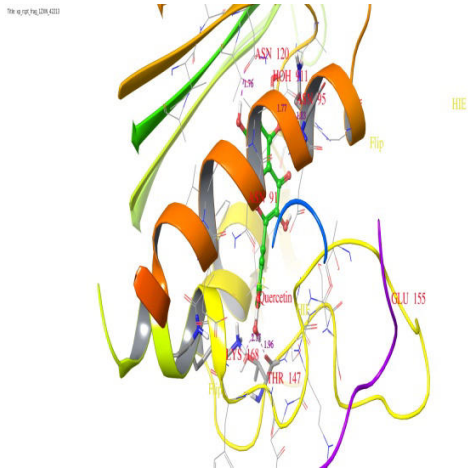


Figure 8 LC-MS spectra of isolated compound (CH2)

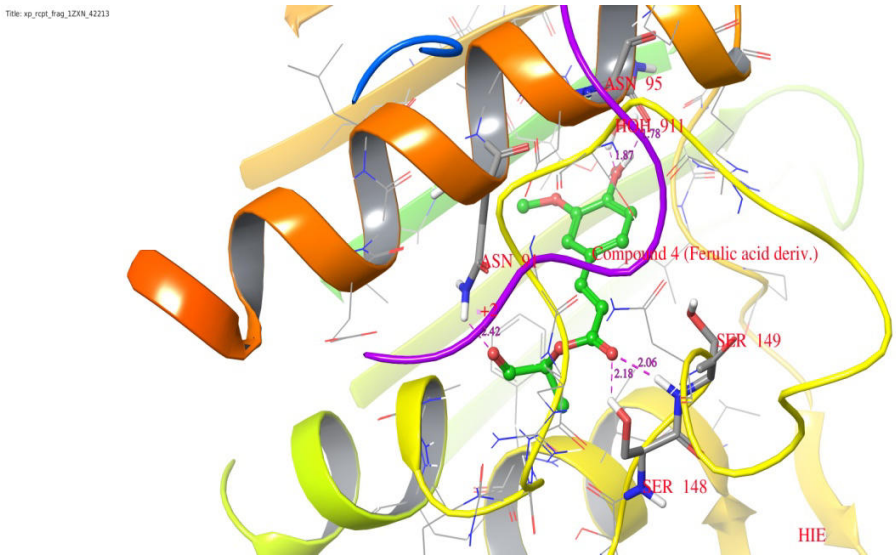
Docking postures of molecules on 1ZXN Enzyme Target



Docking Posture of Camptotechin

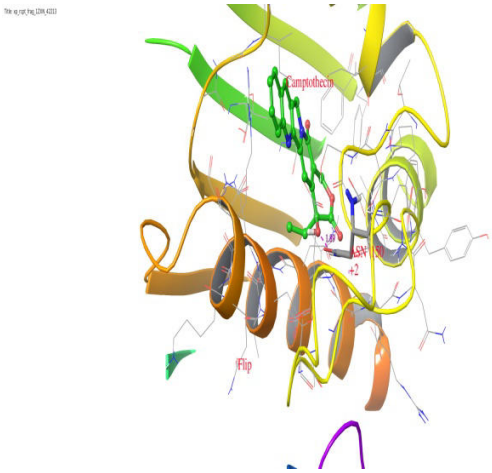


Docking Posture of Quercitin

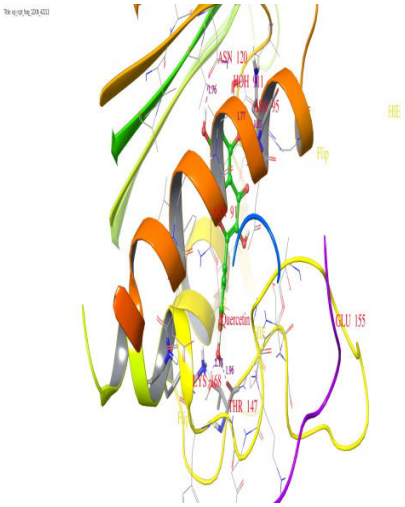


Docking Posture of NOVEL COMPOUND (CH2)

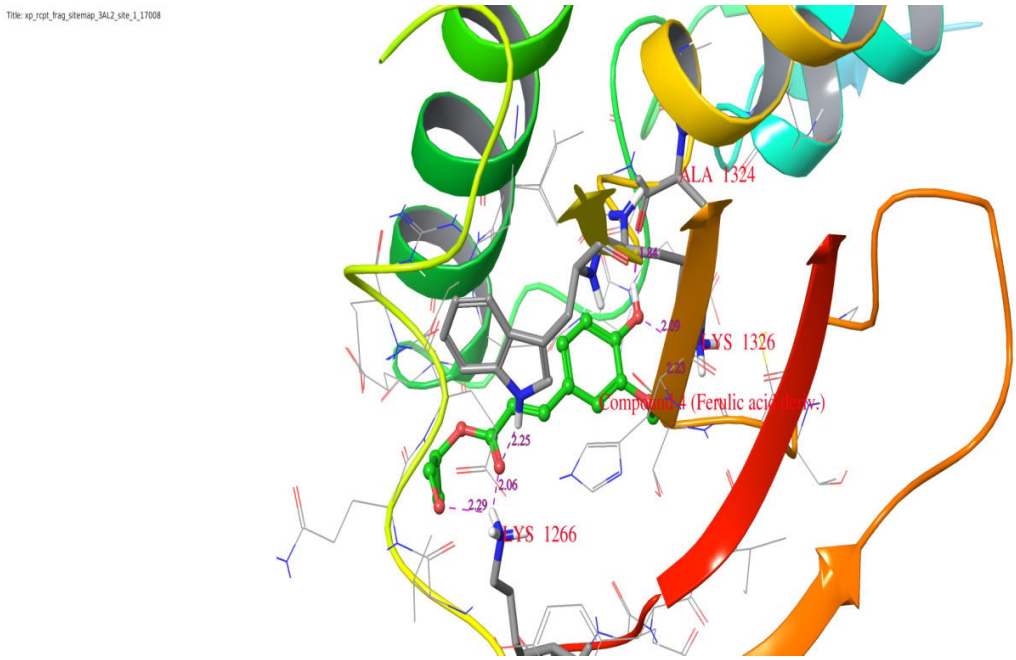
Docking postures of molecules on 3AL2 Enzyme Target



Docking posture of Camptotechin



Docking posture of Quercitin



Docking Posture of Novel compound (CH2)