CHAPTER 9

ISOLATION OF PROBABLE BIOACTIVE COMPOUNDS FROM ROOT BARK OF *CROTON CAUDATUS* GEISELER

9.1 Introduction

Plant extracts from aerial part contain compounds of wide polarity and structural types, while most mono and sesquiterpenoids are volatile compounds acting as antiherbivore agents, attractants, repellants etc., di and higher terpenoids, alkaloids etc are mostly non-volatile constituents having diverse functions (***Walton and Brown, 1999***).

Alkaloids constitute a diverse and impressive class of natural products found mainly in plants, with low-molecular weight nitrogen-containing basic structures. Over 20,000 different alkaloids have been described, illustrating their structural and biosynthetic diversity compared to those of other secondary metabolites have traditionally been used as antitussives, sedatives, purgatives and other treatments for various ailments in the form of medicinal plant extracts.

The alkaloids such as taxol, vincristine and vinblastine (anticancer), ajmalicine and serpentine (anti-hypertensive), ajmaline (anti-arrhythmic), sanguinarine and berberine (antimicrobial), noscapine (antitussive and potentially anti-neoplastic), papaverine (vasodilator), and (+)-tubocurarine (muscle relaxant) are the highly successful in pharmaceuticals industry (***Wani et al., 1971, Jonson et al., 1963, Jordan et al., 1991, Kutcan et al., 1991***).

Paclitaxel (Taxol), produced by Taxus spp., is a diterpenoid alkaloid and an important anticancer agent used as a first line treatment for several types of cancer, including breast, ovarian and non-small cell lung cancer, and has also shown efficacy against AIDS related Kaposi sarcoma (***Cragg and Newman, 2005***). Camptothecin derivatives are used clinically as antitumor alkaloids. At present, camptothecin and its related compounds are obtained by extraction from intact plants (***Saito et al., 2001***).

The isolation of two complex alkaloids-vincaleukoblastine (VLB) and leurocristine (LC) from the pantropical plant *Catharanthus roseus* (L,) G. Don *(Vinca rosea* L,) (Apocynaceae)-initiated a resurgence of interest in this area. The success of leurocristine, termed a "miracle drug" in treating acute childhood lymphocytic leukemia and a wide variety of other human neoplasms is well documented (***Taylor, 1968).***

Berberine has a long history of use for eye infections. In one study that looked at effectiveness in treating trachoma, berberine was more effective than sulfacetamide in eradicating *Chlamydia trachomatis* from the eye and preventing relapse of symptoms (***Babbar, 1982, Mohan, 1982***). Berberine is an isoquinoline alkaloid, present in roots and stem-bark of *Berberis* species. Berberine is chief alkaloid from roots and stem-bark of *Berberis* species. It is manufactured mostly from roots of *B.aristata* (5% in roots and 4.2% in stem-bark), *B. Petiolaris* (0.43%), *B.vulgaris, B. aquifolium, B. thunbergii* and *B. asiatica* (***Watt, 1972, Nandkarni, 1976, Chopra et al., 1996),*** *C. teeta* (rhizome 8-9%) and *Hydrastis Canadensis* ***(Gruenwald et al., 2000).***

Berberine is capable of inhibiting growth and endogenous platelet-derived growth factor synthesis in vascular smooth muscle cells after *in vitro* mechanical injury (***Liang, et al., 2008***). Berberine was also found to inhibit the intestinal secretory response of *Vibrio cholerae* and *Escherichia coli* enterotoxins without causing histological damage to the intestinal mucosa (***Sack, 1982***). Berberine has definite potential as drug, since it possesses diverse pharmacological properties.

Camptothecin is an indole terpenoid alkaloid produced by Camptotheca acuminata (Nyssaceae). It is used as an anticancer drug (review by ***Ulukan and Swan, 2002***) because of its ability to inhibit DNA topoisomerase I (***Kjeldsen et al., 1992***).

Therefore, we have decided to isolate the bioactive compounds from the root bark of *Croton caudatus* Geiseler.

9.2: Materials and Methods

9.2.1 Isolation and Purification of the compound obtained from the root bark of *Croton caudatus* Geiseler:

The root bark of *Croton caudatus* Geiseler were clearly washed with water, shade dried (air dried) and powdered. The powdered root barks (300g) were defatted with petroleum ether. After wasing with petroleum ether the residue were extracted exhaustively with 200ml distilled methanol by using soxhlet apparatus. The extract was filtered through cotton followed by vacuum suction. The solvent was then removed by distilling at 20-30^{0}C to give 4.5g of a reddish brown sticky semi-solid mass.

The reddish brown sticky semi- solid compound obtained from methanol extract showed five spots on TLC with hexane and ethyl acetate (9:1). It was hence, chromatographed over a column of silica gel (100-200 mesh, 50g column 25cm) using pure hexane and ethyl acetate. The progress of the elution was monitored by TLC examination of eluent fraction. Chromatographically similar fractions were combined and solvents removed by distillation to yield a whitish yellow sticky semi-solid compound (S.D.1) and a white solid compound (S.D.3) when the concentration of the above eluent has been alter to 8:2.

The various fractions collected from the column chromatography of the methanol extract of root bark of *Croton caudatus* Geiseler, according to their increasing order of polarity are shown in the table below:

Table 9.2.1: The various fractions collected from the column chromatography of the methanol extract of root bark of *Croton caudatus* Geiseler.

Eluent	Fraction No	Remark
Hexane:Ethyl acetate(9:1)	**10-15**	**Yellow semi-solid(S.D.1)**
Hexane:Ethyl acetate(8:2)	**20-24**	**White solid(S.D.3)**

Fractions 16-19 was rejected for irrecoverable component overlapping.

9.3: Results and Discussions

The extract of the roots bark of *Croton caudatus* Geiseler was subjected to column chromatography using silica gel (100-200 mesh) as stationary phase and eluted with hexane and ethyl acetate (95:5). From the column the different chromatographically similar fractions were collected and solvent is evaporated under reduced pressure and the residue is collected. The residue from fraction 10-15 is then

subjected to TLC to know the purity of the compound and then to study GC-MS with proper pre-determined solvents. The GC indicated that the compound to have a very high purity (peak at 14.98min) although it contains two more peaks within 2.64 min.

9.3.1: Spectral analysis of S.D.1

The spectral analyses of S.D.1 were done with reference to FT-IR, MS and ^{1}H NMR. The data recorded in the above memtioned spectral analysis is presented below.

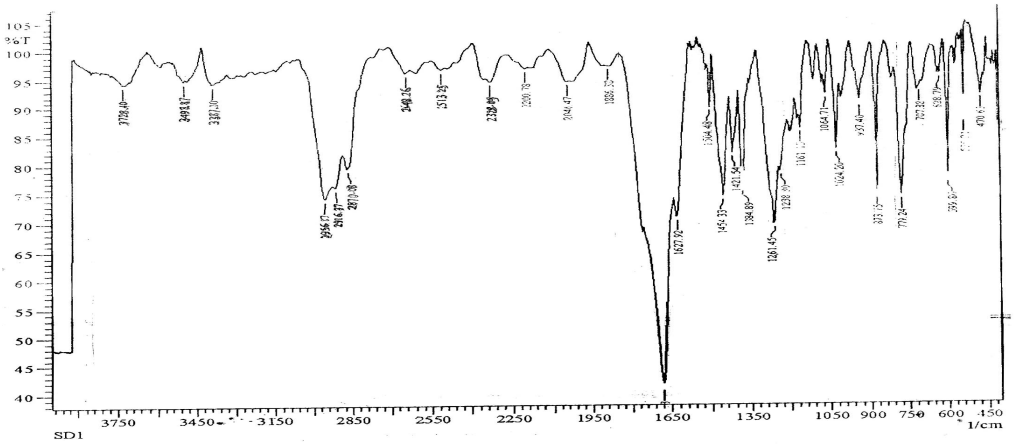


Fig.9.3.1(a): FT-IR Spectrum of isolated compound (S.D.1) from root bark of *Croton caudatus* Geiseler.

Table 9.3.1(a): Assignments of functional group corresponding to the bands obtained from FR-IR of isolated compound (S.D.1) from *Croton `caudatus* Geiseler

Wave Numbers(cm^{-1})	Characteristic Peaks
3498	γ_{O-H} of -OH
2956	γ_{C-H} of $-CH_3$ and $-CH_2-$
2916	Asymmetrical CH- str.in CH_2 gp
1627	$\gamma_{C=C}$
1680	$\gamma_{C=O}$(Conjugated)

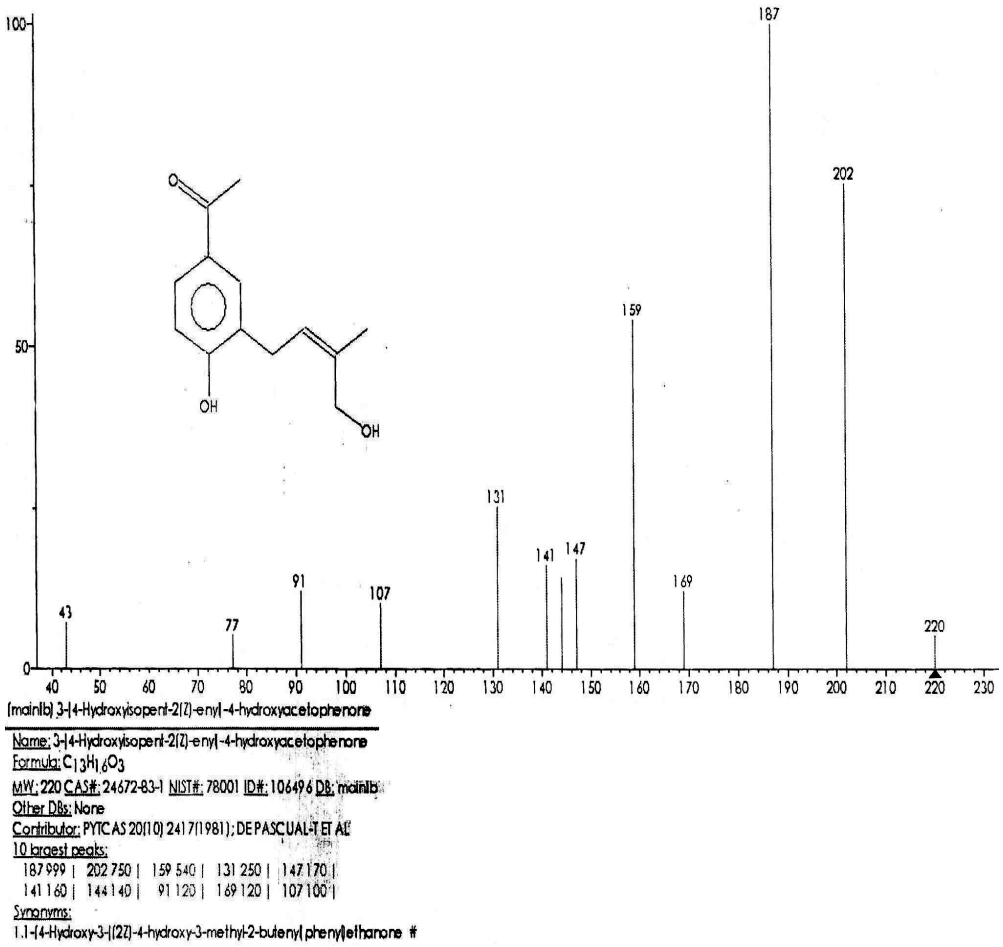


Fig.9.3.1 (b): Mass Spectrum of isolated compound (S.D.1) from root bark of *Croton caudatus* Geiseler.

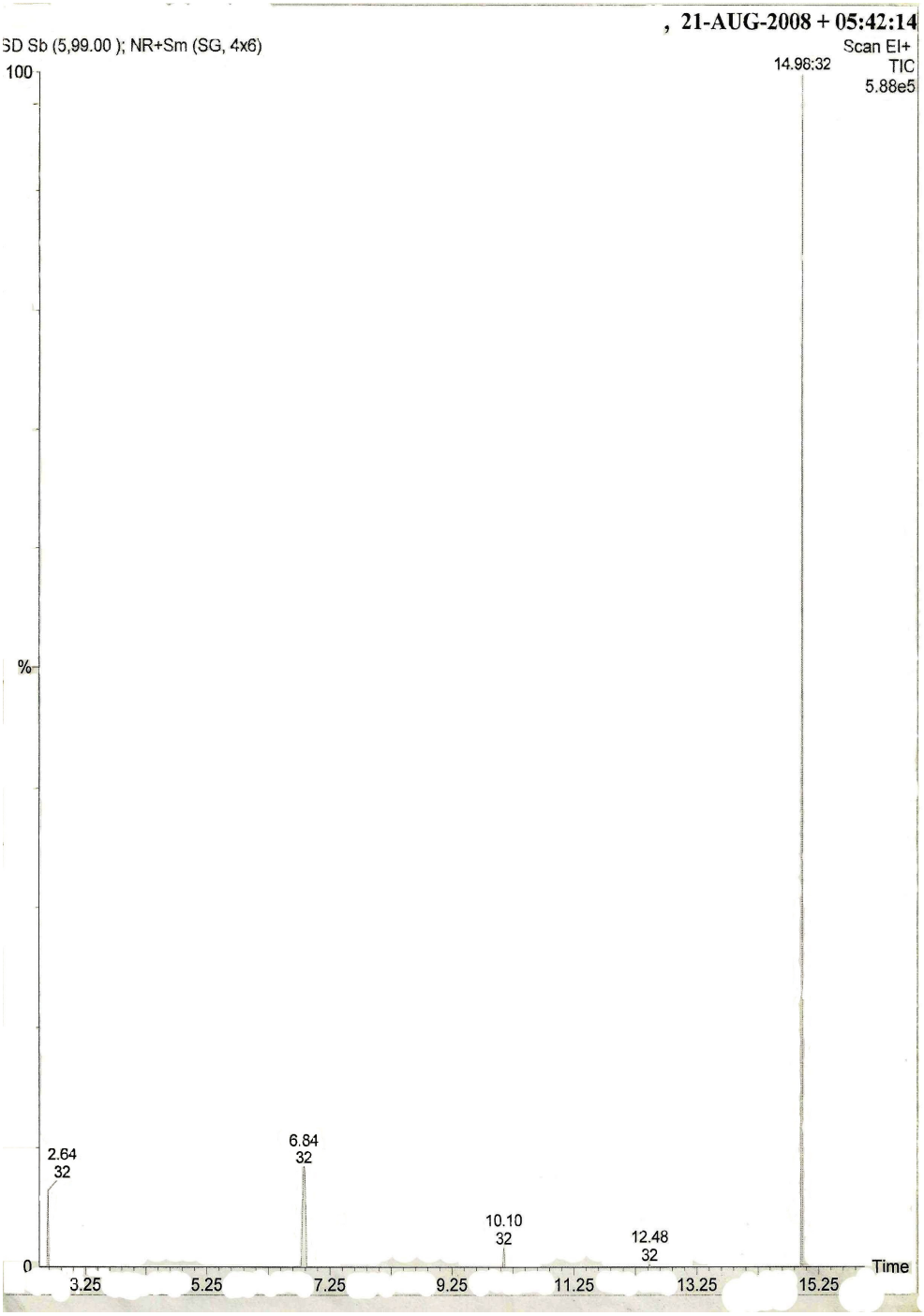


Fig.9.3.1(c): GC Spectra of isolated compound (S.D.1) from root bark of *Croton caudatus* Geiseler.

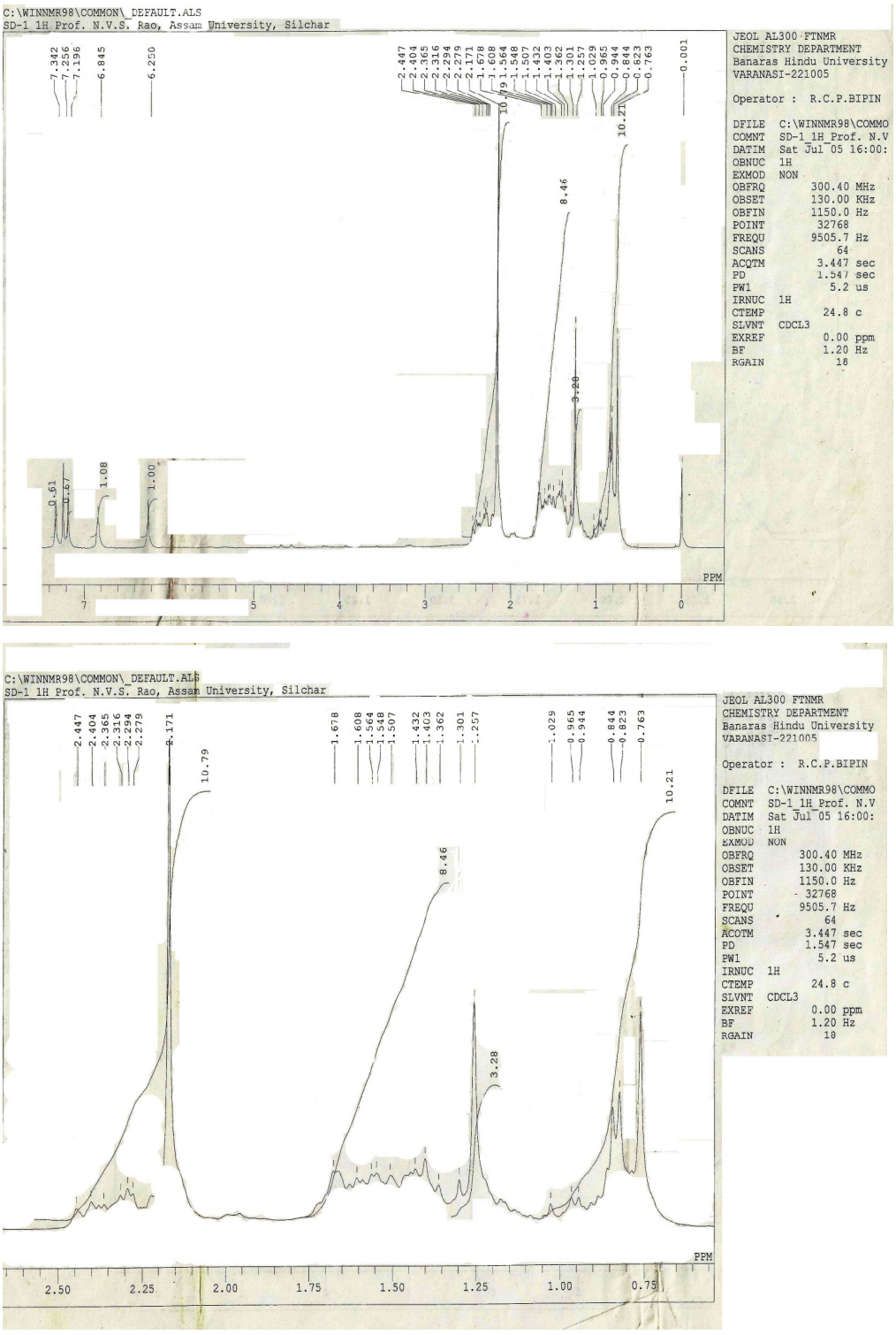
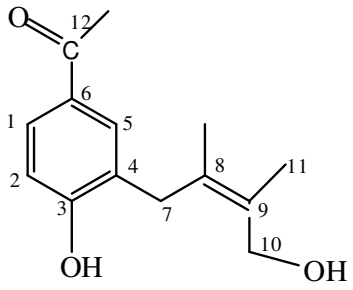


Fig.9.3.1(d): ^{1}H NMR Spectra of isolated compound (S.D.1) from root bark of *Croton caudatus* Geiseler.

Assignments of functional group corresponding to the bands obtained from ^{1}H NMR of isolated compounds from *Croton caudatus* Geiseler (S.D.1).



In the ^{1}H NMR spectral analysis (***Sharma, 1986, Kalsi,1995***, ***Silverstein et al., 1991, Finar, 1975, Morrison and Boyd, 2011,***), the peaks at ð 7.34ppm is for H-1, 6.8 ppm(H-2),6.2 ppm and 4.5 for aromatic proton,1.7ppm(H-7),7.2 ppm(H-5), 4.5ppm for alcoholic proton The peak at 2.1 ppm and 1.4ppm for methyl proton.

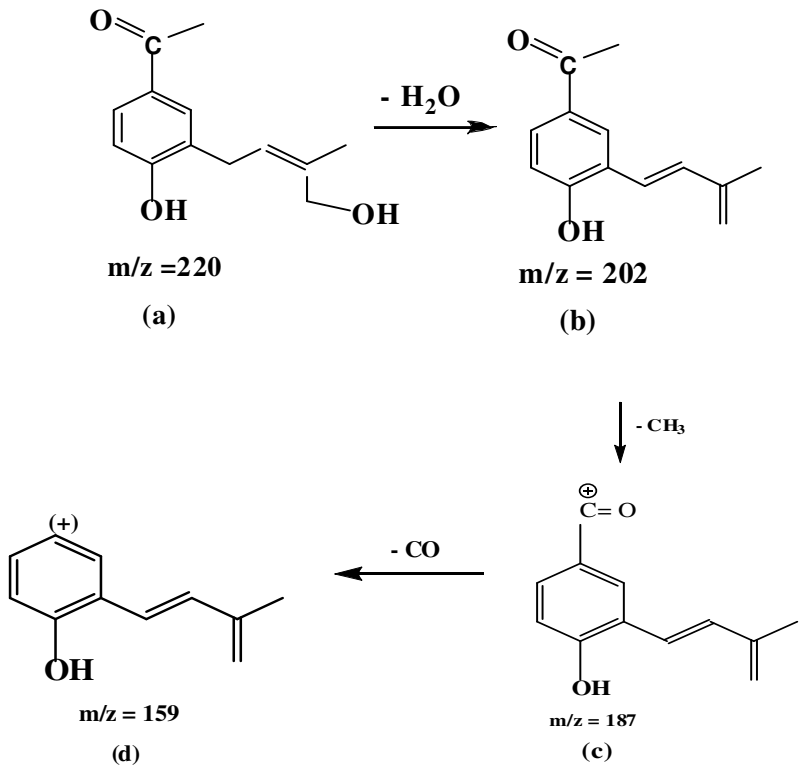
The isolated compound is a known compound, (ID 4524652) . ***(Pascual-T et al., 1981).***

Molecular formula: $C_{13}H_{16}O_3$

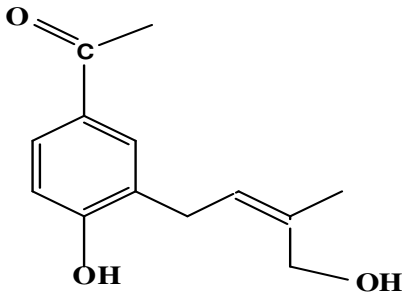
Molecular mass: 220

The proposed MS fragmentations are shown below:

(i)



Thus, the structure of the compound is proposed as:



1-[4-hydroxy-3[(2z)-4-hydroxy-3-methyl-2-butenylphenyl] ethanone.

9.3.2: Spectral analysis of S.D.3

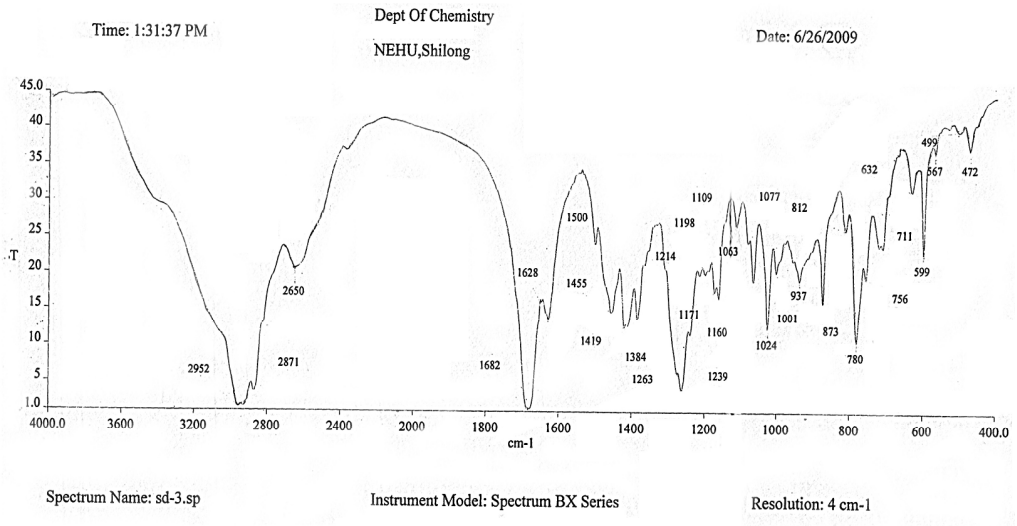


Fig.9.3.2(a): FT-IR Spectrum of isolated compound (S.D.3) from root bark of *Croton caudatus* Geiseler.

Wave Numbers(cm^{-1})	Characteristic Peaks
2952	CH_3- CO str.
2871	CH_3 str
2650	C- C str
1682	Cyclic CO-N-CO str
1419	N- CH_3 str
1384	C= C str
1109	Cyclic five membered ring
1171,1160	Cyclic six membered ring
1024	Cyclopropane ring

Table 9.3.2(a): Assignments of functional group corresponding to the bands obtained from FT-IR of isolated compounds from *Croton caudatus* Geiseler (S.D-3).

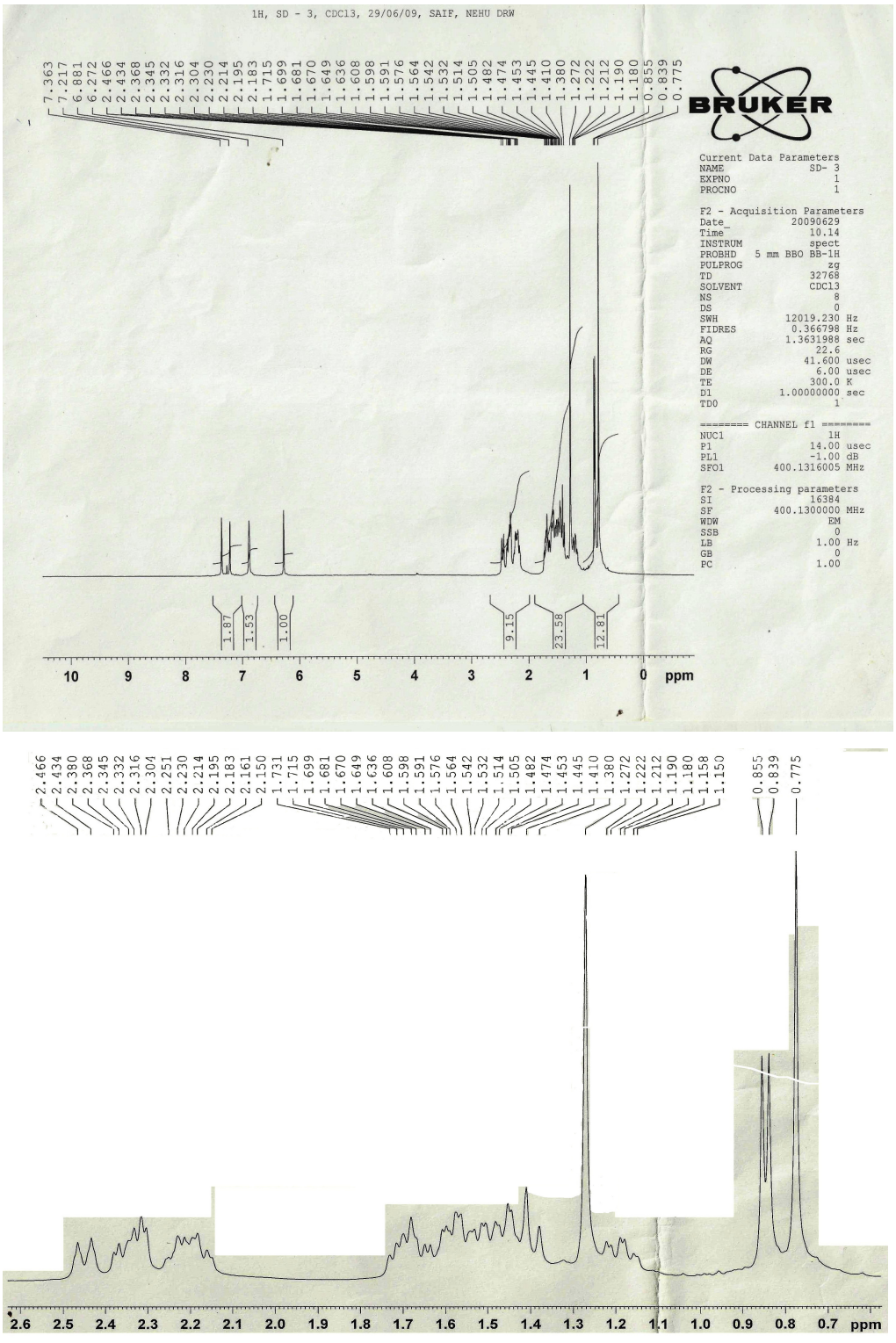
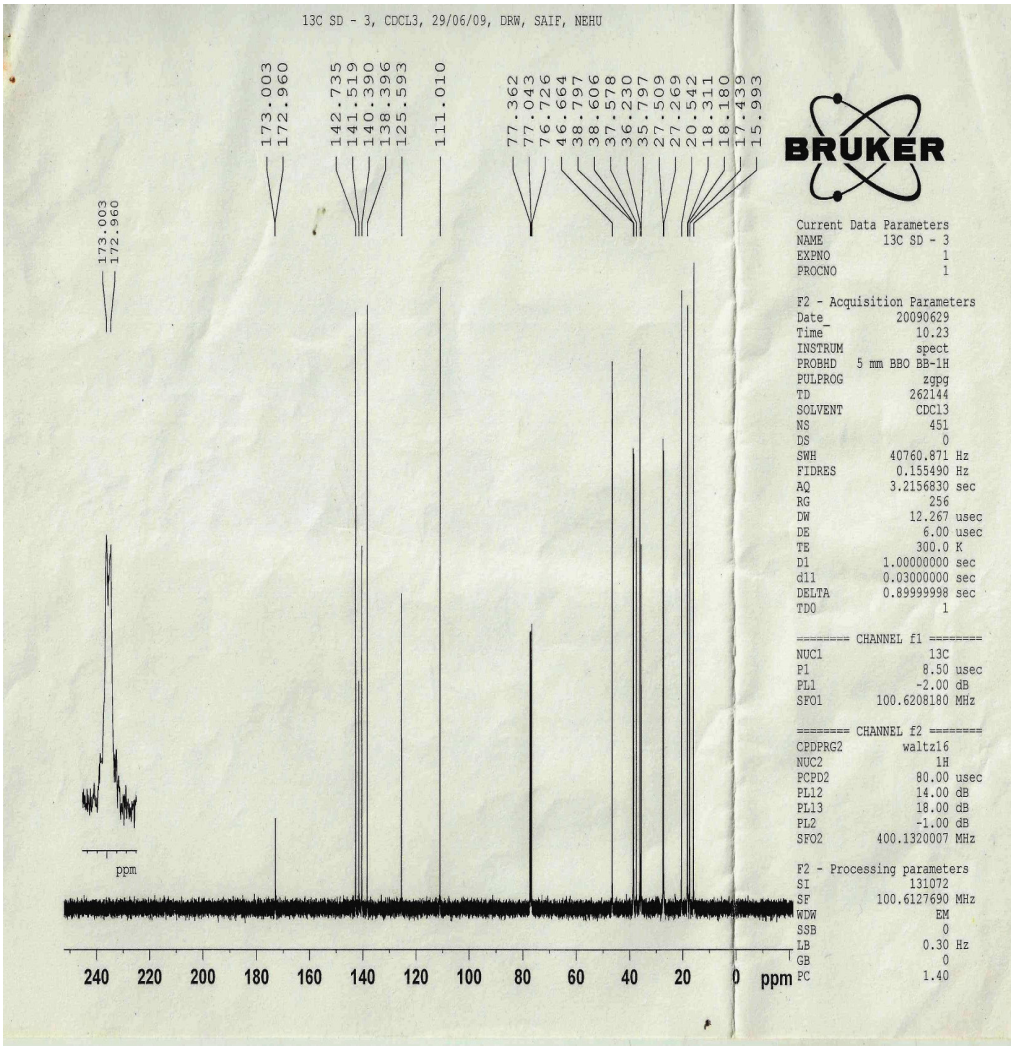


Fig.9.3.2(b): ^{1}H NMR Spectra of isolated compound (S.D.3) from root bark of *Croton caudatus* Geiseler.

In the [1]H NMR spectral analysis (***Sharma, 1986, Kalsi 1995***, ***Silverstein et al., 1991, Finar, 1975, Morrison and Boyd, 2011,***), the peaks at ð 1.56ppm is for H-1. The peaks at ð 1.54 ppm (H-2), 1.53 ppm (H-3), ð 1.51 ppm (H-4), ð 1.50 ppm (H-5), ð 1.19ppm (H-6), ð 1.21ppm (H-7), ð 1.22 ppm (H-8),ð 1.27 ppm (H-9), ð 1.38 ppm (H-10),ð 1.41ppm (H-11),ð1.44 ppm (H-12),ð1.18ppm (H-13),ð1.47ppm (H-15), ð1.45 ppm (H-16), ð0.83ppm (H-18),ð0.855ppm (H-19),ð 2.19ppm (H-21), ð 1.48ppm (H-22), ð 2.21ppm (H-25), ð 0.77ppm (H-26-28) respectively.



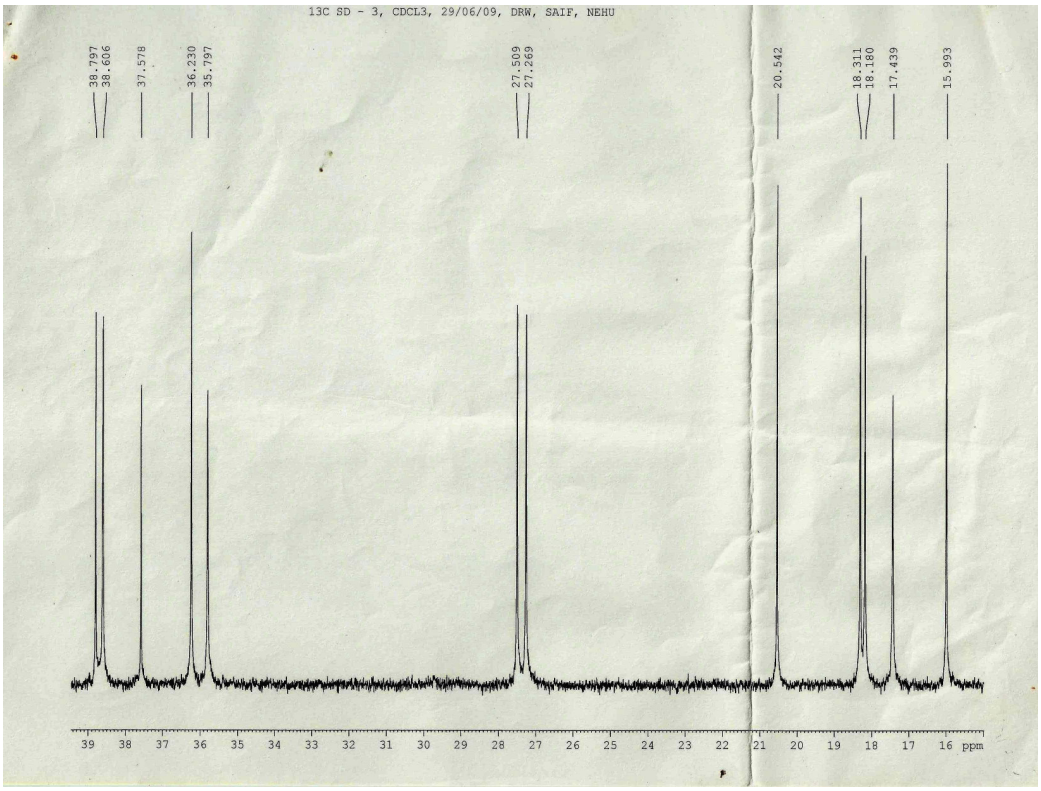


Fig.9.3.2(c): ^{13}C Spectra of isolated compound (S.D.3) from root bark of *Croton caudatus* Geiseler.

In the ^{13}C spectral analysis,(***Sharma, 1986, Kalsi, 1995***, ***Silverstein et al., 1991, Finar, 1975, Morrison and Boyd, 2011,***), δ_C,46.66(C-1), 27.26(C-2,3), 36.23(C-4,8,9), 20.54(C-5,17), 27.50(C-6,7), 37.57(C-10), 35.79(C-11,16), 38.79(C-12), 17.43(C-13), 141.51(C-14), 38.60(C-15),18.18(C-18), 18.31(C-19), 138.39(C-20), 140.39(C-21), 125.59(C-22), 111.01(C- 23,24), 142.73(C-25), 15.99(C- 26,27,28).

DEPT 135 and DEPT 45 support the presence of CH_3 at ðppm as in ^{13}C spectra for C- 18, 19, 21, 25.

DEPT 90 supports the presence of CH at ðppm as in^{13}C spectra for C- 1, 4.8, 9, 10, 12, 14, 22, and 26.

DEPT 135 supports the presence of CH_2 by their negative signal at ðppm as in ^{13}C spectra for C- 2, 3, 6, 7,11, 15, 16, 27, 28.

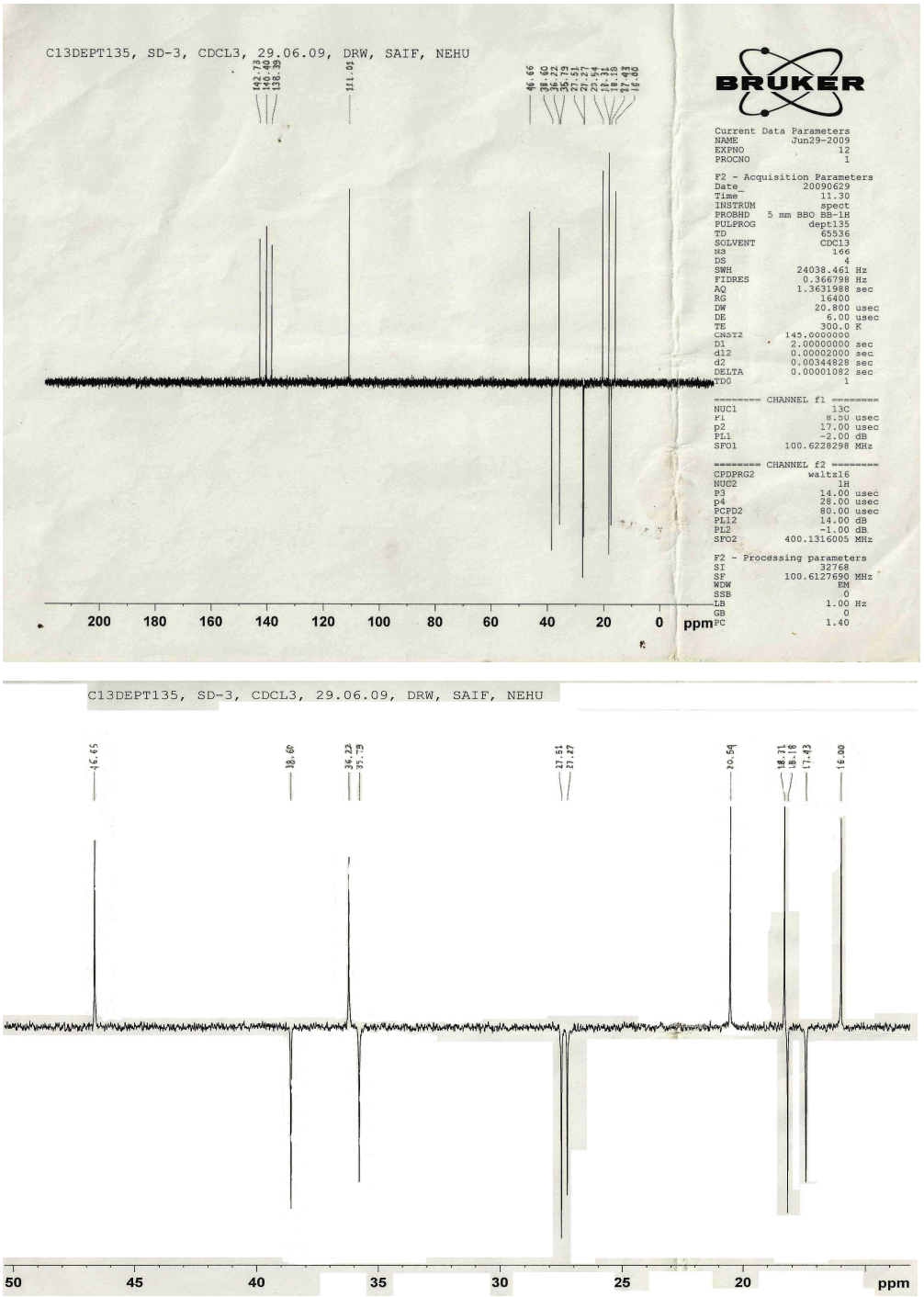
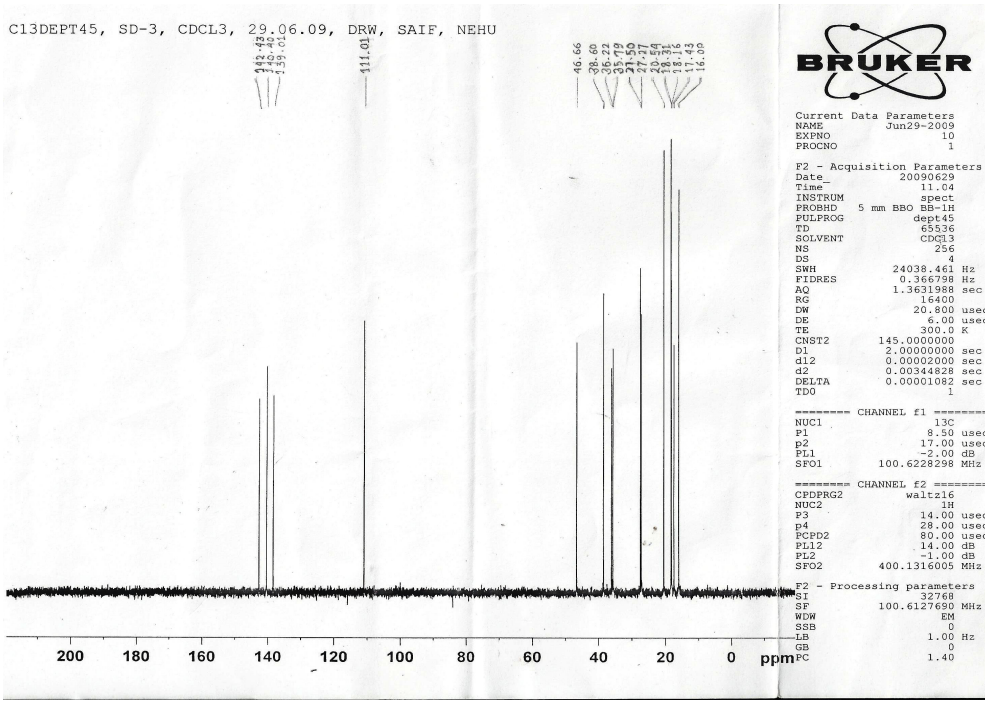


Fig.9.3.2(d): DEPT 135 Spectra of isolated compound (S.D.3) from root bark of *Croton caudatus* Geiseler.



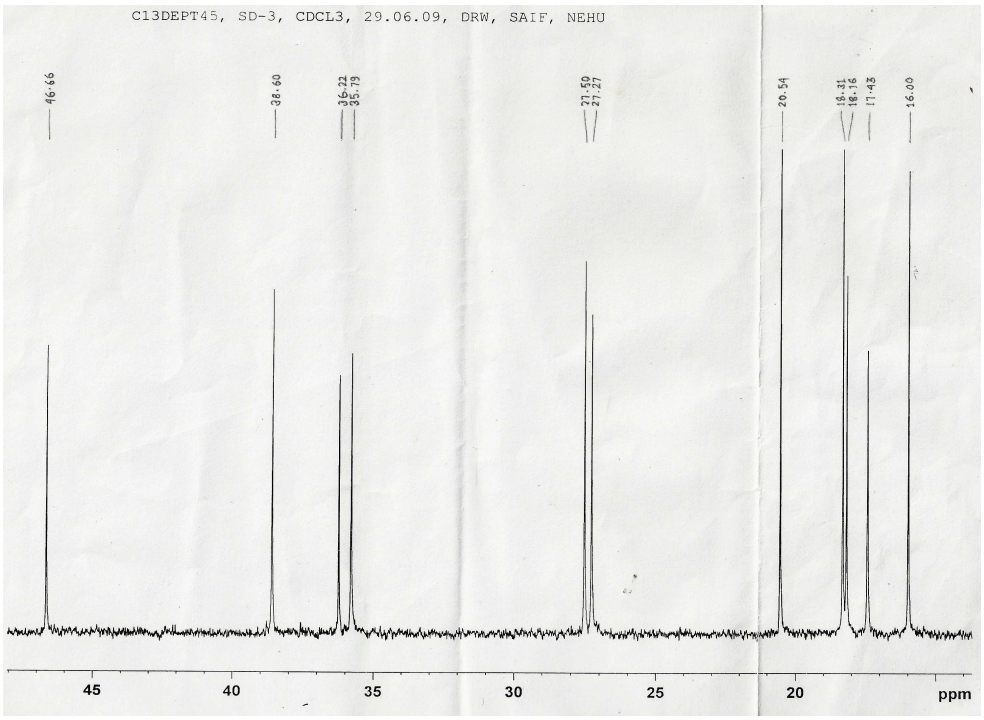


Fig.9.3.2(e): DEPT 45 Spectra of isolated compound (S.D.3) from root bark of *Croton caudatus* Geiseler.

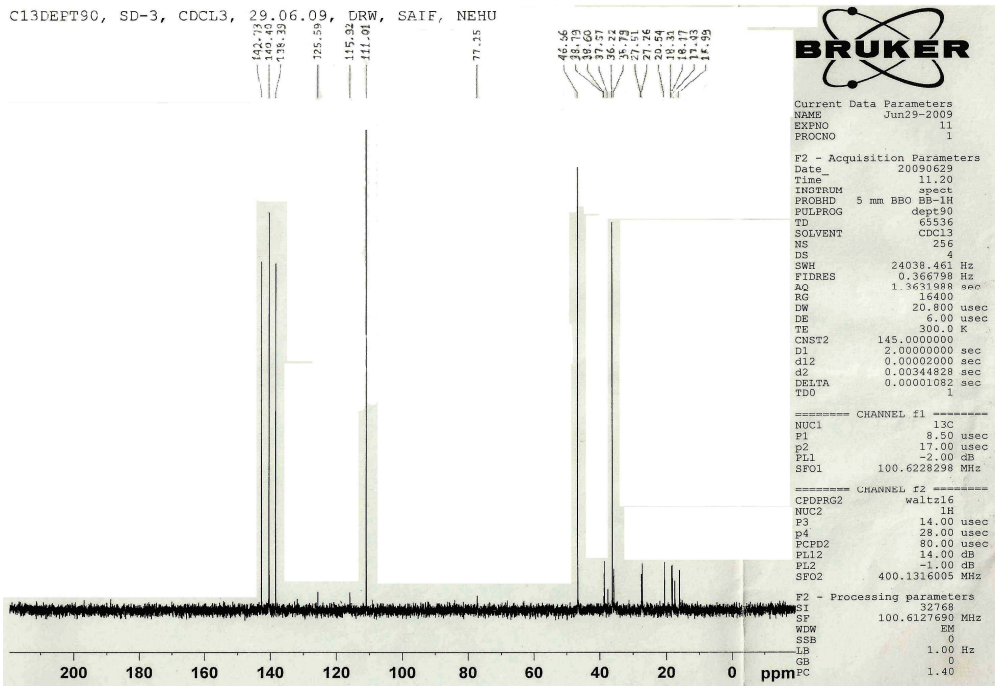


Fig.9.3.2(f): DEPT 90 Spectra of isolated compound (S.D.3) from root bark of *Croton caudatus* Geiseler.

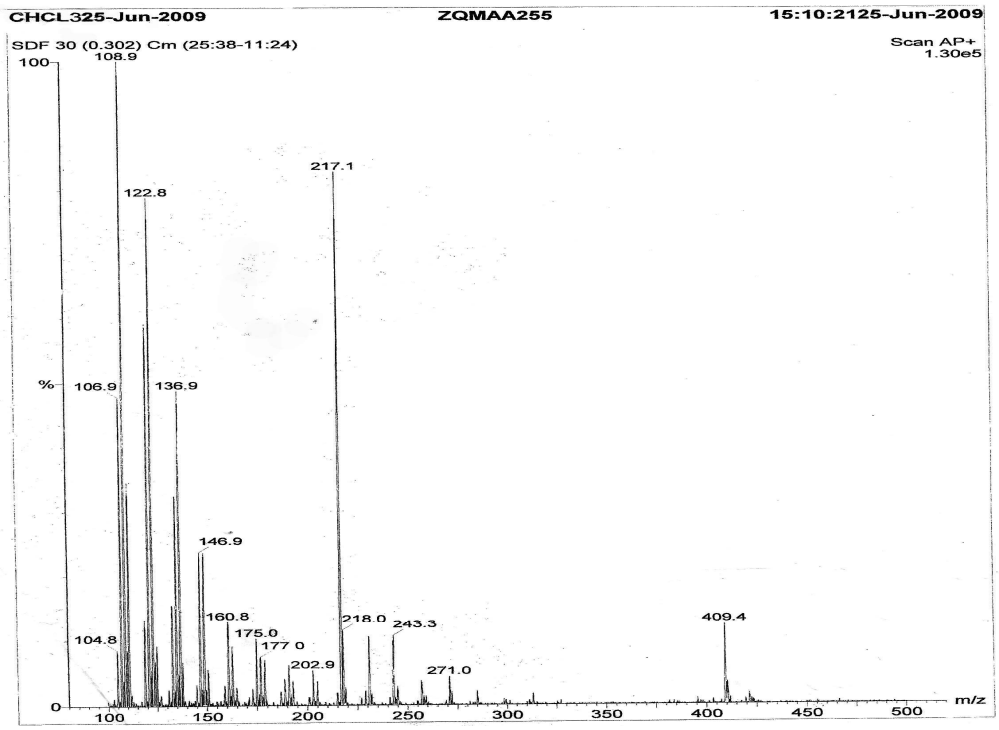
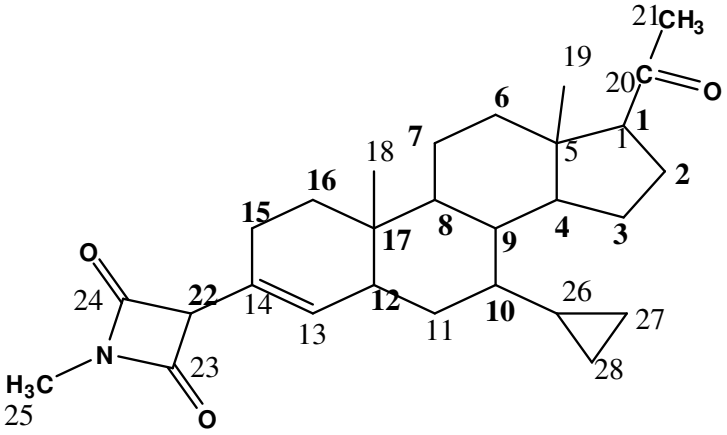
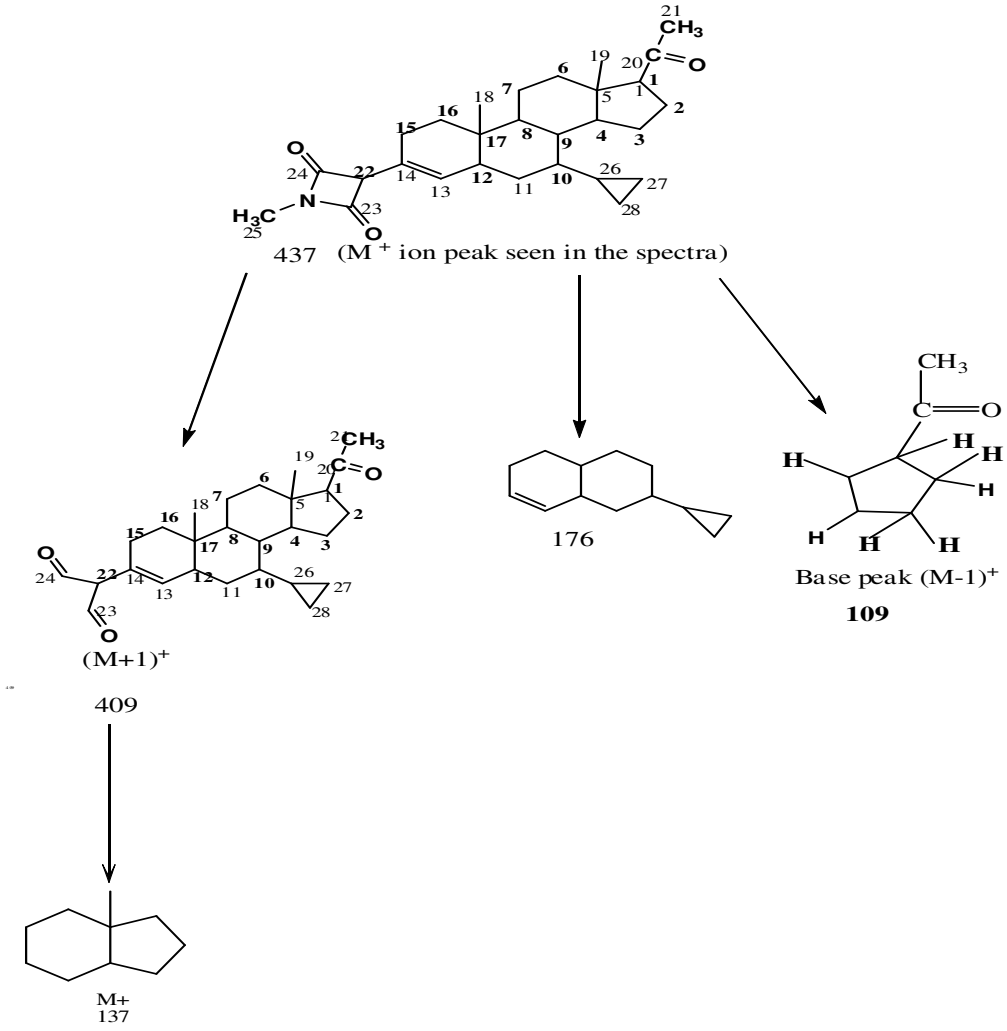


Fig.9.3.2(g): Mass Spectra of isolated compound (S.D.3) from root bark of *Croton caudatus* Geiseler.

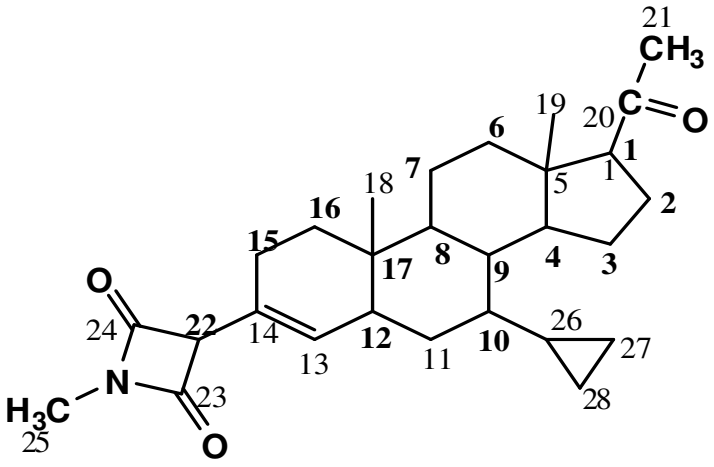
From the above spectral analysis, the proposed structure is



The proposed MS fragmentations are shown below:



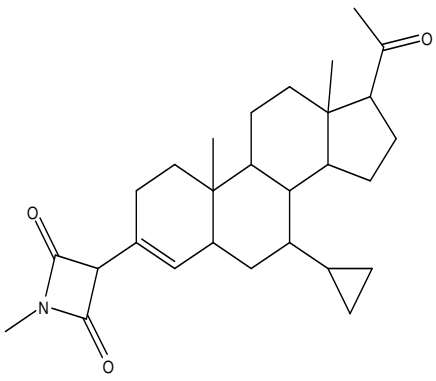
Thus, the tentative structure of the compound is proposed as



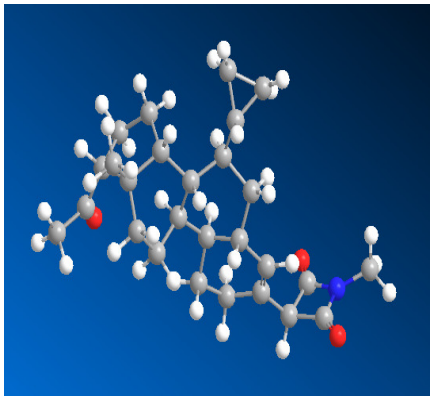
3-(17-acetyl-7-cyclopropyl-10,13-dimethyl-2,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)-1-methylazetidine-2,4-dione

9.4 Pharmacological co-relation of the isolated compound (S.D.3) and Silymarin:

In QSAR (Qulitative Structural Analysis Relationship) analysis, the calculated total energy value of the isolated compound (S.D.3) is found to be 103.372 kcal/mol whereas that of Silymarin is 8.740 kcal/mol.

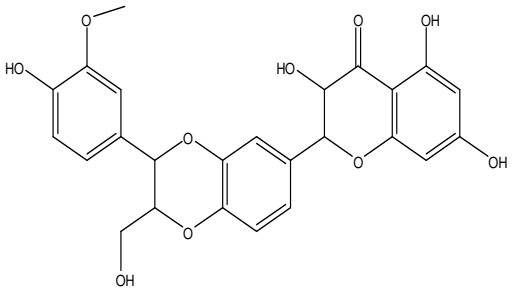


S.D.3



3D structure of isolated compound

Total Energy: 103.372 kcal/mol



Structure of Silymarin 3D structure of Silymarin

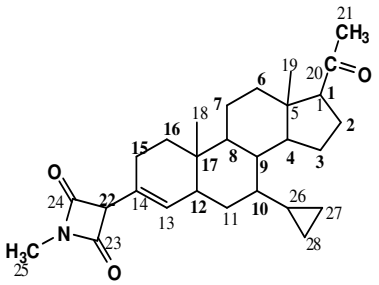
Total Energy: 8.740 kcal/mol

Although the total energy value of the isolated compound (S.D.3) is different from that of silymarin, the other parameters mainly enzyme inhibitor, nuclear receptor ligand and GPCR Ligand(Glutamate Pyruvate Co-valent Receptor Ligand) values of the isolated compound were much more than that of silymarin.

Table 9.4.1 Comparison of certain properties between the isolated Compound (S.D.3) and the standard drug Silymarin:

Properties	**Isolated Compound**	**Silymarin**
LogP	4.635	1.465
TPSA	54.451	155.147
GPCR ligand	0.31	0.07
Ion channel modulator	0.18	-0.05
Kinase inhibitor	0.40	0.01
Nuclear receptor ligand	0.63	0.16
Protease inhibitor	0.19	0.02
Enzyme inhibitor	0.53	0.23

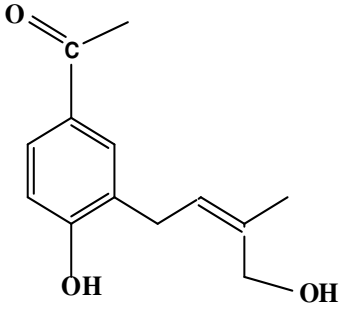
From the above table, the other parameters such as LogP value, TPSA (Total polar surface area), ion channel modulator, kinase inhibitor and protease inhibitor values of the isolated compound were also higher than that of Silymarin. So, the higher hepatoprotective activity of the methanol extract of root bark of *Croton caudatus* Geiseler than that of Silymarin is probably due to the presence of these compound S.D.3 i.e.



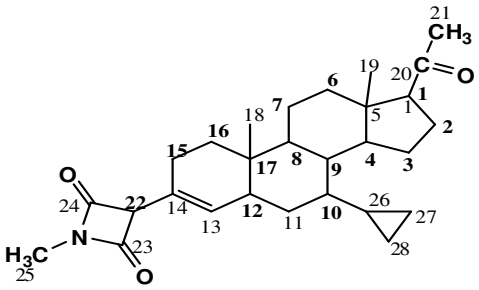
3-(17acetyl-7cyclopropyl-10,13-dimethyl-2,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta [a] phenanthren-3-yl)-1-methylazetidine-2, 4-dione.

9.5: Conclusion

From the above spectral analysis, the compounds isolated from methanol extract of root bark of *Croton caudatus* Geiseler were found to be



(S.D.1)



(S.D.3)

1-[4-hydroxy-3[(2z)-4-hydroxy-3-methyl-2-butenylphenyl] ethanone, an already isolated known compound **(S.D.1) and** 3-(17acetyl-7cyclopropyl-10,13-dimethyl-2,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta [a] phenanthren-3-yl)-1-methylazetidine-2, 4-dione **(S.D.3).**

The pharmacological co-relation of the isolated compound (S.D.3) with that of Silymarin reveals that the isolated compound (S.D.3) obtained from the root bark of *Croton caudatus* Geiseler shows better hepatoprotective activity as compared to Silymarin, the standard drug which is also corroborated by the study of the biochemical parameters with the methanol extract of the above mentioned root bark.

9.6 Bibliography

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