CHAPTER 10

ISOLATION OF TRITERPENOIDS FROM STEM BARK OF *Phyllanthus acidus* (L) Skeels

10.1 Introduction

The number of higher plants on this planet is estimated to be 250,000 with a lower level at 25,000 (***Cronquist, 1981 and 1988***). Only 6% of these plants have been screened for their biological activity and about 15% have been evaluated phytochemically. Medicinal herbs are considered to be a chemical factory, which contains multitude of chemical compounds like alkaloids, saponins, glycosides, steroids, resins, terpenoids and oils etc.

As early as Charak-Sushruta era (600 BC) vegetable oil (unsaturated) was generally recommended as an adjunct to treatment of Madhumcha with total withdrawl of animal fats (saturated***) (Grover et al., 2001).***

Oils from sweet almond, apricot, peach kernal, sesame seed, soyabean, sunflower, avocado and jajoba are stable to oxidation and having no smell of their own find use in perfumes as carrier oil(dilutent) ***(Vankar, 2004, Bhat, 2005***).

Phytosterols isolated from plants are useful as raw material for the synthesis of hormones and related pharmaceuticals and cosmetics and as additive to thermoplastic resins used in the manufacture of rubber materials including tyres ***(Abidi et al., 1999, Vlahakis et al., 2000, Hetherington et al., 1999).***

Linoleic acid controls arteial and heart diseases and it is antirephrotoxic ***(Kaleem, 2004***). High monosaturated fatty acid (MUFA) diet lowers both plasma cholesterol and TAG concentration

(Kris-Etherton et al., 1999). Tocopherols could be responsible for plants potent antioxidant activity.These natural constituents of oils are more active than the synthetic racemic ± α-tocoperol ***(Walton and Brown, 1999).***

The triterpene groups of compounds include sterols and triterpenes, which can accumulate as glycosides (saponins) in extensive amounts in plants ***(Sparg et al., 2004).*** Several thousand terpenes and terpenoids occur in many genera of higher plant (***Devon and Scott, 1972, Darnley-Gibbs, 1974).*** Ganoderic acids C and D isolated from Ganoderma lucidum, species used against different immune implicated diseases, such as allergy, inflammation and cancer ***(Giner-Larza et al., 2000, Ríos, 2008).***

Various triterpenes were assayed as inhibitors of induced DTH reactions, especially those isolated from the immunomodulator plants *Tripterygium wilfordii* ***(Li and Weir, 1990),*** *Cayaponia tayuya* ***(Ríos et al., 1990),*** *Astragalus membranaceus* ***(Ríos and Waterman, 1997***) and *Glycyrrhiza glabra* ***(Kroes et al., 1997)***. Celastrol (tripterine) ***Ali et al. (2006)*** isolated a new triterpenoid with anticomplement activity, namely podocarpaside from *Actaea podocarpa* (Ranunculaceae), which inhibited human complement activity.

Triterpenoids are metabolites of isopentenyl pyrophosphate oligomers and represent the largest group of phytochemicals. It has been estimated that more than 20,000 triterpenoids exist in nature (***Liby et al., 2007***). Triterpenoids are biosynthesized in plants by the cyclization of squalene, a triterpene hydrocarbon and precursor of all steroids (***Phillips et al., 2006***). Triterpenoids are used for medicinal purposes in many Asian countries for antiinflammatory, analgesic,

antipyretic, hepatoprotective, cardiotonic, sedative and tonic effects (***Ovensná et al., 2004, Huang, 1993***). Recent studies have confirmed some of the aforesaid pharmacological properties of several triterpenoids along with additional biological activities including antioxidant, antimicrobial, antiviral, antiallergic, antipruritic, antiangiogenic and spasmolytic activity (***Sultana and Ata, 2008, Shah et al., 2009***). An increasing number of triterpenoids have been reported to exhibit cytotoxicity against a variety of cancer cells without manifesting any toxicity in normal cells (***Setzer et al., 2003, Laszczyk, 2009, Petronelli et al., 2009)***. They also demonstrate antitumor efficacy in preclinical animal models of cancer (***Laszczyk, 2009***, ***Petronelli et al., 2009***).

Therefore, we have decided to isolate triterpenoids from stem bark of *Phyllanthus acidus* (L.)Skeel.

10.2: Materials and Methods

10.2 (a) Isolation and Purification of the compound obtained from the stem bark of *Phyllanthus acidus* (L) Skeels:

The stem bark of *Phyllanthus acidus* (L)Skeels were collected, clearly washed with water, shade dried (air dried) and powdered.The powdered root bark (500g) were defatted with petroleum ether.After wasing with petroleum ether the residue were extracted exhaustively with 300ml distilled methanol by using soxhlet apparatus. The extract was filtered through cotton followed by vacuum suction. The solvent was then removed by distilled off 20-30^{0}C to give 6.5g of a brownish white semi-solid mass.

10.3: Results and Discussions

The extract of the stem bark of *Phyllanthus acidus* (L)Skeels was subjected to column chromatography using silica gel (100-200 mesh) as stationary phase and eluted with hexane and ethyl acetate (90:10). From the column the different chromatographically similar fractions were collected and solvent is evaporated under reduced pressure and the residue is collected. A small fraction of the residue is then subjected to TLC to know the purity of the compound.

Table10.3: The various fractions collected from the column chromatography of the methanol extract of stem bark of *Phyllanthus acidus* (L)Skeels

Eluent	Fraction No	Remark
Petroleum ether:Ethyl acetate(9:1)	**10-15**	**Yellow semi-solid(C.A.1)**
Petroleum ether:Ethyl acetate(8:2)	**20-25**	**Yellow semi-solid(C.A.1)**

The fractions collected were 10-15 and there was no indication of any compound in fraction no. 16 onwards.So, the concentration of eluent has been changed to 8:2 and fractions collected from 20-25 indicated TLC spots for compound.

10.3.1: The spectral analysis of C.A.1 was done with reference to FT-IR, MS and ^{1}H NMR.

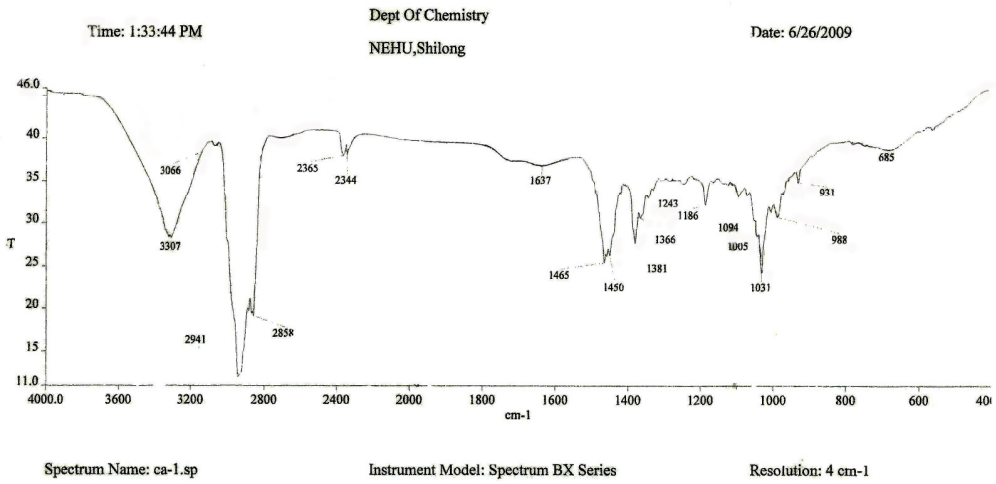

Fig.10.3.1(a): FT-IR Spectrum of isolated compound (C.A.1) from stem bark of *Phyllanthus acidus* (L)Skeels.

Table 10.3.1(a): Assignments of functional group corresponding to the bands obtained from FT-IR of isolated compounds from stem bark of *Phyllanthus acidus* (L)Skeels.

Wave Numbers(cm^{-}	Characteristic Peaks
3307	γ O-H str(H-bonded)
3066	γ C-H str,attached with $CH=CH_2$
2858	γ C-Hstr
1637	γ C=O srt
1465	$-CH_3$ str
1450	$\gtrdot CH_2$ str
1381	$(CH_3)_3C$- str
1366	CH_3-CO- deformation
1243	$(CH_3)_3C$-skeleton vibration
1186	2^0- OH deformation
1094	Cyclohexane ring str
1031	C-O str

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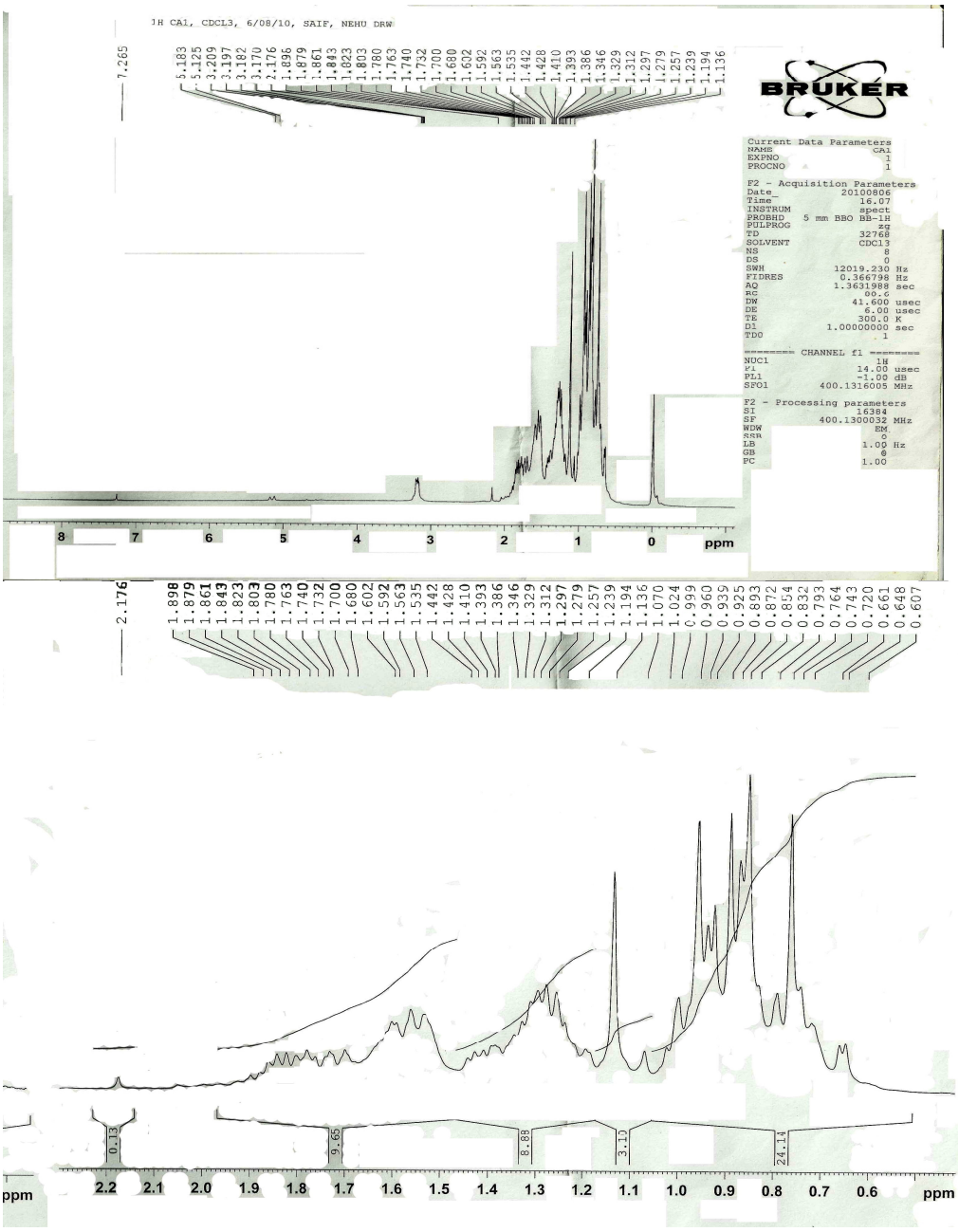
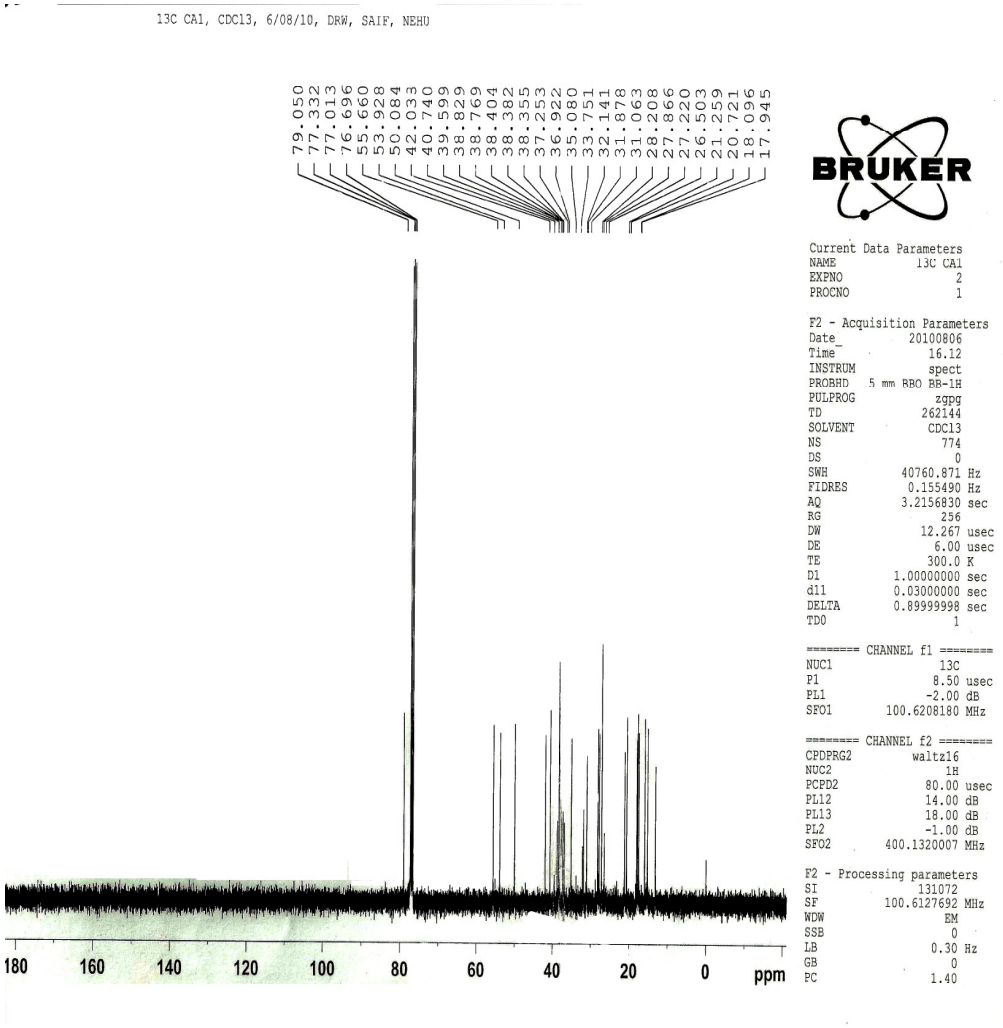


Fig.10.3.1 (b): ^{1}H NMR Spectrum of isolated compound (C.A.1) from stem bark of *Phyllanthus acidus* (L)Skeels.

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 0.93ppm is for H-1. The peaks at ð0.96 ppm (H-2), ð0.99

ppm (H-3), ð1.42 ppm (H-5),ð 1.44 ppm (H-6), ð0.72 ppm (H-7),ð 1.31 ppm (H-8), ð0.74 ppm (H-9), ð1.53 ppm (H-10,11), ð0.76 ppm (H-12),ð1.32 ppm (H-13),ð0.79ppm (H-14), ð1.56ppm (H-15),ð1.59 ppm (H-16), ð0.83ppm (H-17), ð1.34 ppm (H-18),ð0.85ppm (H-19), ð1.60ppm (H-20),ð1.68ppm (H-21),ð0.89 ppm (H-22),ð1.25 ppm (H-23),ð 3.170ppm (H-24),ð1.41ppm (H-26),ð1.70ppm (H-29), ð0.92 ppm (H-30),ð0.87ppm (H-31),ð 3.18ppm (H-34), ð 3.19ppm (H-35),ð 3.20 ppm (H-36).



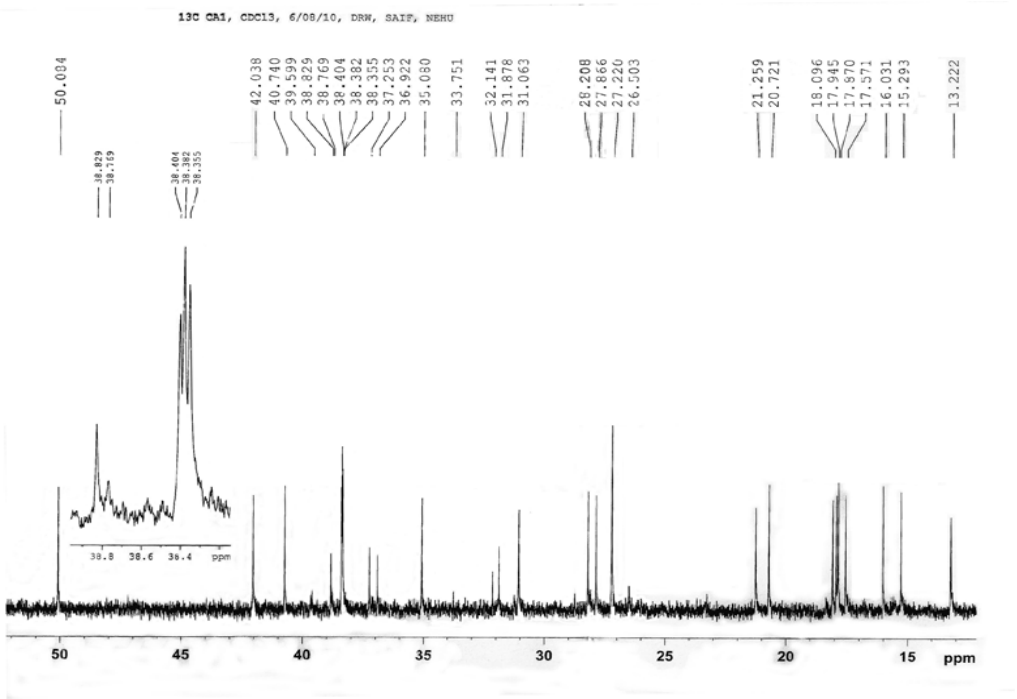


Fig.10.3.1.(c): ^{13}C Spectrum of isolated compound (C.A.1) from stem bark of *Phyllanthus acidus* (L) Skeels.

In the ^{13}C spectral analysis, (***Sharma, 1986, Kalsi, 1995***, ***Silverstein et al., 1991, Finar, 1975, Morrison and Boyd, 2011,***), δ_C, the peak at 27.22 ppm is forC 1-3, 27.86 ppm (C-4), 32.14ppm (C-5), 33.75ppm (C-6),13.22ppm (C-7), 21.25ppm (C-8,13,18), 15.29ppm (C-9), 35.08ppm (C-)10, 36.92ppm (C-11),16.03ppm (C-12),17.57 ppm (C-14), 37.25ppm (C-15), 38.35 ppm (C-16), 17.87ppm(C-17), 17.94 ppm (C-19), 38.38 ppm (C -20), 38.40ppm (C-21),18.09 ppm (C-22), 38.76ppm (C-23), 39.59 ppm (C-24), 55.66 ppm (C-25), 50.08 ppm (C-26), 38.82ppm (C-27), 40.74 ppm (C-28), 20.72ppm (C-29), 31.06 ppm (C-30), 28.20ppm (C-31), 53.92ppm (C-32), 31.87ppm (C-33).

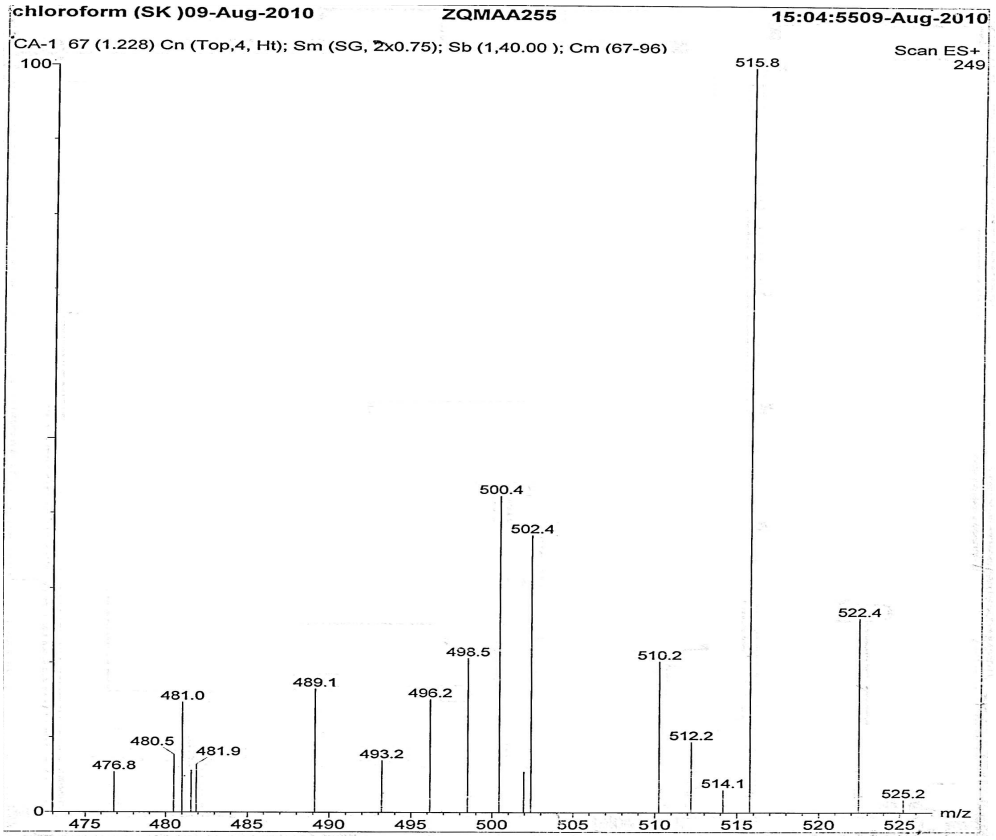
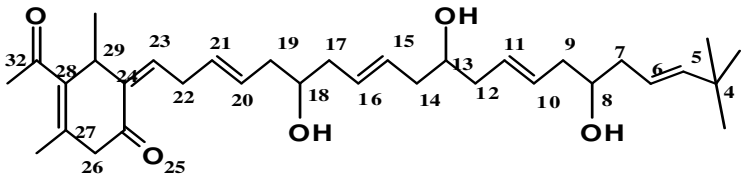
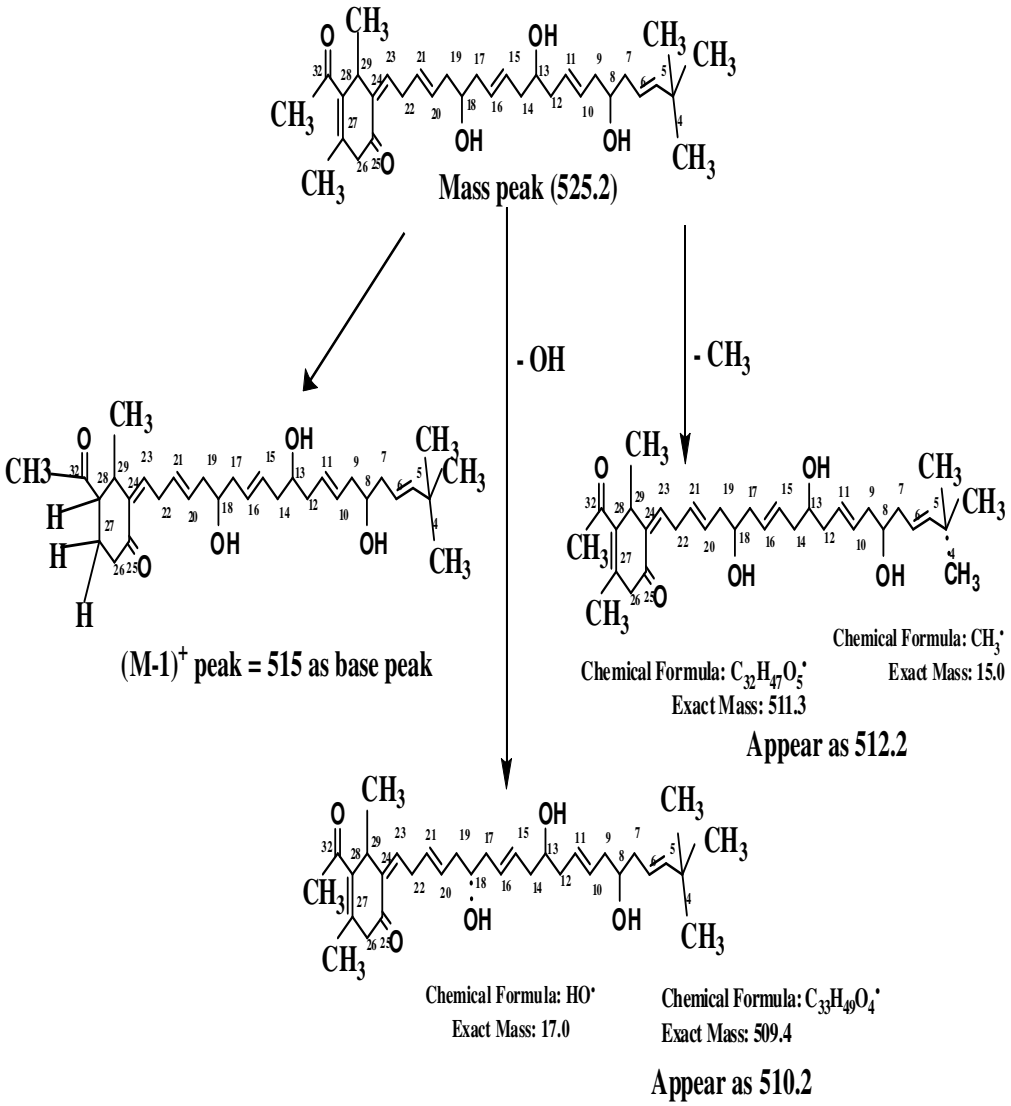


Fig.10.3.1(d): Mass spectra of isolated compound (C.A.1) from stem bark of *Phyllanthus acidus* (L)Skeels.

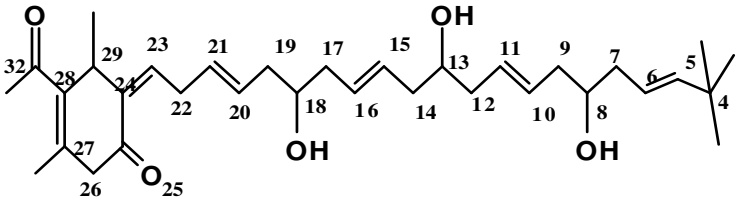
From the above spectral analysis (FT-IR, ^{1}H-NMR, ^{13}C and mass spectra) the proposed structure is



The proposed MS fragmentations are shown below:



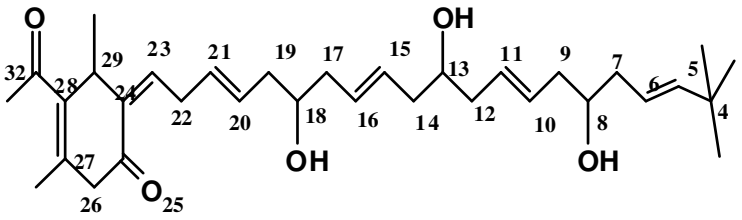
Thus, the tentative structure of the compound is proposed as



(Z)-4-acetyl-3,5-dimethyl-6-((3*E*,8*E*,13*E*,18*E*)-6,11,16-trihydroxy-20,20-dimethylhenicosa-3,8,13,18-tetraenylidene)cyclohex-3-enone

10.4: Conclusion

From the above spectral data analysis, the compound isolated from stem bark of *Phyllanthus acidus* (L)Skeels was found to be



(*Z*)-4-acetyl-3,5-dimethyl-6-((3*E*,8*E*,13*E*,18*E*)-6,11,16-trihydroxy-20,20-dimethylhenicosa-3,8,13,18-tetraenylidene)cyclohex-3-enone

For optimization of bioactivity and to know the potency as anti-inflamatory and anticarcinogenic property of the above triterpenoid, further research work is necessary.

10.5: Bibliograpy

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