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Pharmaceutical Sciences
Volume 5 Issue 2, 2025
eISSN No.: 2582-8371

Phytochemical Unveiling of *Dillenia pentagyna Roxb.*: Isolation of Bioactive Metabolites for Cytotoxic Potency

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ABSTRACT

This study explored the phytochemical composition and cytotoxic potential of Dillenia pentagyna Roxb. bark extract through qualitative, quantitative, and spectral analyses. Ethanolic extract (DPBE) yielded 12.23% with significant presence of flavonoids, alkaloids, triterpenoids, and phenolic compounds. Quantitative estimations revealed 62.20 mg GAE/g of total phenolic content and 58.26 mg QUE/g of flavonoids. HPLC fingerprinting and chromatography led to the isolation of bioactive triterpenoids and flavonoids, including lupeol (DPE-1), betulin (DPE-2), betulinic acid (DPE-3), and β-sitosterol (DPE-A1), confirmed via FTIR, NMR, and mass spectrometry. In-vitro cytotoxicity of these compounds was evaluated against HT-29 colorectal and MCF-7 breast cancer cell lines using the MTT assay. Among them, DPE-2 and DPE-3 showed significant anticancer activity with high percent inhibition and lower IC_{50} values. The results suggest these isolated compounds possess promising anticancer potential and validate the traditional use of D. pentagyna in herbal medicine. Further in-vivo and mechanistic studies are required to explore their therapeutic applicability.

Keywords: *Dillenia pentagyna*, *Triterpenoids*, *Cytotoxicity*, *Spectral characterization*, *MTT assay.*

INTRODUCTION

Herbal medicine has long served as a foundation for drug discovery due to its rich source of bioactive secondary metabolites. *Dillenia pentagyna Roxb.*, a plant traditionally used in treating inflammation and cancer, has been insufficiently characterized chemically.[1] The present study was undertaken to identify and isolate active compounds from its bark extract, assess their structural features via advanced spectroscopic techniques, and evaluate their cytotoxic potential against cancer cell lines.[2] The Global Cancer Observatory database predicted that the anticipated new cancer-related cases would be around 21.6 million globally from 2020 to 2025. There will be a 12.1% rise in new cancer cases, with 11.4 million cases in males and 10.2 million in females by 2025. There were an estimated 10 million deaths because of cancer in 2020. According to projected estimates, there will be 47% more cancer cases by 2040, low human development index (HDI) countries will contribute to 95% of cases, and medium HDI countries will contribute to 64% of cases[3-6]. By 2025, it is predicted that there will be 11.3 million cancer-related deaths worldwide[7]. Among Indians, the primary cause of death by cancer of the lips and mouth is due to the practice of chewing betel nuts[8]. In India, cancer of the oral cavity, digestive system, respiratory system, and genital system indicated a higher projected crude cancer rate for the year 2020[9]. The predicted incidence of new cancer cases in India by the various states and Union territories in 2020 was 1.39 million. It grew to 1.42 million in 2021 and 1.46 million in 2022, according to the National

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Cancer Registry Programme of the Indian Council of Medical Research (ICMR). India is expected to burden 29.8 million cancer patients by 2025 report[10-16].
This investigation bridges traditional claims with modern scientific validation.

MATERIALS AND METHODS

Plant Material:

Bark of *Dillenia pentagyna* Roxb. was collected from Central Western Ghats of India. The location is Kulagi Forest Range, Dandeli, in the month of February.

The bark of *Dillenia pentagyna* was shade-dried, powdered, and extracted using ethanol (yield: 12.23%). Preliminary phytochemical screening was conducted for common classes like flavonoids, alkaloids, triterpenoids, and phenols. Quantitative estimation of phenolics and flavonoids was performed using Folin-Ciocalteu and aluminium chloride methods respectively. HPLC fingerprinting and chromatographic separation helped isolate compounds, which were structurally characterized by FTIR, 1H-NMR, 13C-NMR, and mass spectrometry. In-vitro cytotoxicity was tested using the MTT assay against MCF-7 (breast) and HT-29 (colon) cancer cell lines.

IR spectra of isolated compounds were recorded using FT-IR with an ATR instrument (BRUKER Avance, Alpha-T) at SET's College of Pharmacy, Dharwad. ^{1}H NMR, ^{13}C NMR and HPLC fingerprinting analysis of compounds were carried out in an analytical instrumentation facility, Honeychem Pharma Research Private Limited, Benguluru, Karnataka. ESI-MS spectra of isolated compounds were recorded using Waters, QTOF Micromassat Lab Sophisticated Analytical Instrumentation Facility, Punjab University, Chandigarh.

Extraction

Authenticated bark of Dillenia pentagyna Roxb. was shade dried and pulverized in to coarse material. Coarse plant material was cleaned by passing the powder material through 120 mesh sieves to remove any fine dust or powder, and coarse powder was used for extraction. Dried powder of bark was exhaustively extracted with methanol in a soxhlet extractor technique bark extract was concentrated by rotary flash evaporator, under reduced pressure and controlled temperature, followed by freeze drying and stored in desiccator.

In-Vitro Activity

***In vitro* cell viability assay**

Principle of assay: This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the Orange water soluble substrate 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide. It is a tetrazolium-based compound (MTT) used in cell viability assays to measure mitochondrial activity in living cells.

Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

PROCEDURE

Experimental Procedure

1) MCF7 Cells were incubated at a concentration of 1 × 10 4cells/ml in culture medium for 24 h at 37°C and 5% CO2.

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2) Cells were seeded at a concentration (100µl) 10^4cells/well) in 100µl culture medium and 6.25, 12.5, 25, 50, 100 µg/ml of Samples into micro plates respectively (tissue culture grade, and 96 wells).
3) Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture.
4) Cell cultures were incubated for 24 h at 37°C and 5% CO2 in CO2 incubator.
5) After incubation, the medium was completely removed and Added 20µl of MTT reagent (5mg/ml PBS).
6) After addition of MTT, cells incubated for 4 hours at 37°C in CO2 incubator.
7) Observed the wells for formazan crystal formation under microscope. The orange MTT was reduced to soluble orange formazan product by viable cells.
8) After removing the medium completely. Added 200µlof DMSO (kept for 10 min) and incubate at 37^0C (wrapped with aluminum foil).
   Triplicate samples were analyzed by measuring the absorbance of each sample by a microplate reader at a wavelength of 550 nm.

RESULTS

The extract revealed rich phytochemical content, especially flavonoids and triterpenoids. Quantitative analysis showed high levels of total phenols and flavonoids. HPLC and spectral analysis led to the identification of lupeol, betulin, betulinic acid, and β-sitosterol. Compounds DPE-2 and DPE-3 showed significant cytotoxic effects with high percentage inhibition and IC_{50} values near 60 µg/mL against MCF-7 cells. Structural data validated their identity and therapeutic relevance.

Preliminary qualitative phytochemical analysis:

The percentage yields of bark extracts obtained from of Dillenia pentagyna Roxb. (DPBE) is depicted in the Table 1. The results of preliminary qualitative phytochemical screening of DPBE were performed as per the reported methods, to detect the various classes of phytoconstituents such as carbohydrates, Flavonoids, Alkaloids, Steroids Triterpinoids, Tannins and Phenolic compounds. The results of phytochemicals analysis are presented in Table 2.

Figure 1 Ethanolic bark extract of Dillenia pentagyna

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Table 1 Percentage yield and physical characteristics of bark extract of Dillenia pentagyna Roxb.

Sl. No	Ethanolic bark extract of *Dillenia pentagyna* (DPBE)	
1	Colour	Reddish brown
2	Odour	Characteristic
3	Consistency	Crystilline
4	Yield (%w/w)	12.23

Table 2 Preliminary phytochemical analysis of bark extract of *Dillenia pentagyna* Roxb.

Sl. No	Phytoconstituents	DPBE
1	Carbohydrates	+
2	Tannins and Phenolic compounds	+
3	Flavonoids	+
4	Steroids	+
5	Triterpenoids	+
6	Alkaloids	+

(+)=Present, (–)=Absent

Quantitative Determination of Secondary Metabolites

Estimation of Total Phenolic Content:

The total phenolic content was determined using Folin-Ciocalteau method. Phenolic content was calculated from the regression equation of the standard plot ($y = 0.0045x + 0.0005$, $R^2 = 0.9991$) and is expressed as Gallic acid equivalents. The total Phenol content present in DPBE is 62.20 mg GAE/g.

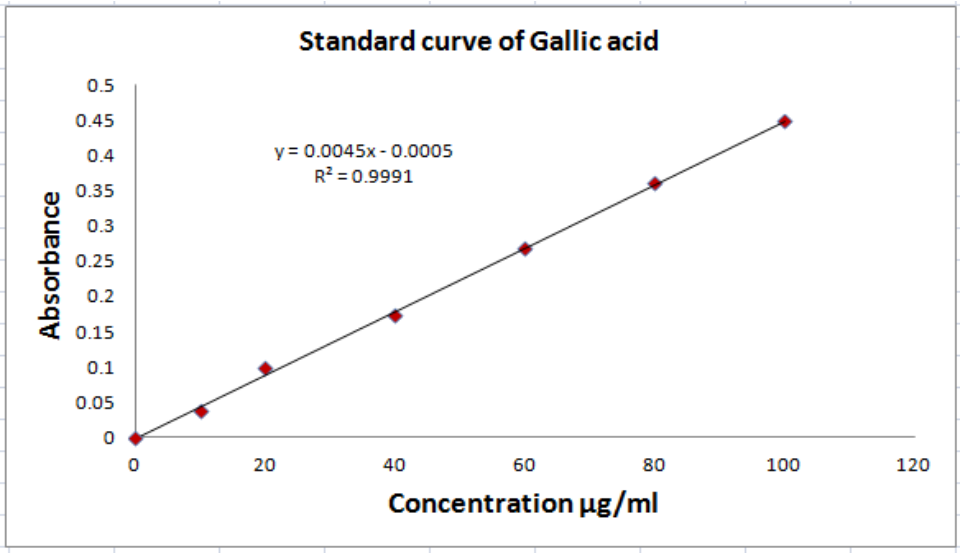

Figure 2 Standard curve for quantification of total phenolic content

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Estimation of Total Flavonoid Content

The total flavonoid content was determined using aluminium chloride method. Flavonoid content was calculated from the regression equation of the standard plot (y = 0.0036x + 0.0068, R^2 = 0.9983) and is expressed as quercetin equivalents. The total flavonoid content present in DPBE is 58.26 mg QUE/g.

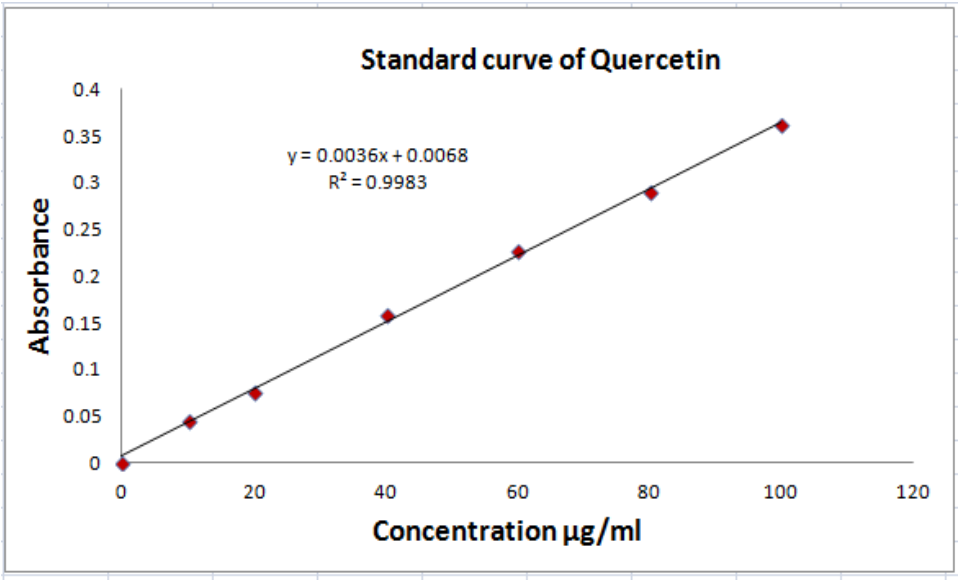

Figure 3 Standard curve for quantification of total flavonoid content

Table 3 Percentage yield of defatted extract and acetone soluble and insoluble fractions

Extracts and Fractions	Yield	
	Grams	Percentage w/w
Ethanolic extract	226.35gms	12.23
Defatted Extract	7.5gms	3.33
Acetone soluble fraction	59gms	29.05
Acetone insoluble fraction	141gms	70.05

Defatted Extract

Acetone soluble fraction

Acetone insoluble fraction

Figure 4 Defatted Extract and Acetone soluble and insoluble fractions

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eISSN No.: 2582-8371

HPLC Finger Printing Analysis

The acetone-soluble fraction was subjected to the HPLC fingerprinting analysis to identify the number of phytoconstituents present in the fraction. Seven peaks were observed in the chromatogram. This was taken as the bases for the isolation of compounds from the acetone-soluble fraction.

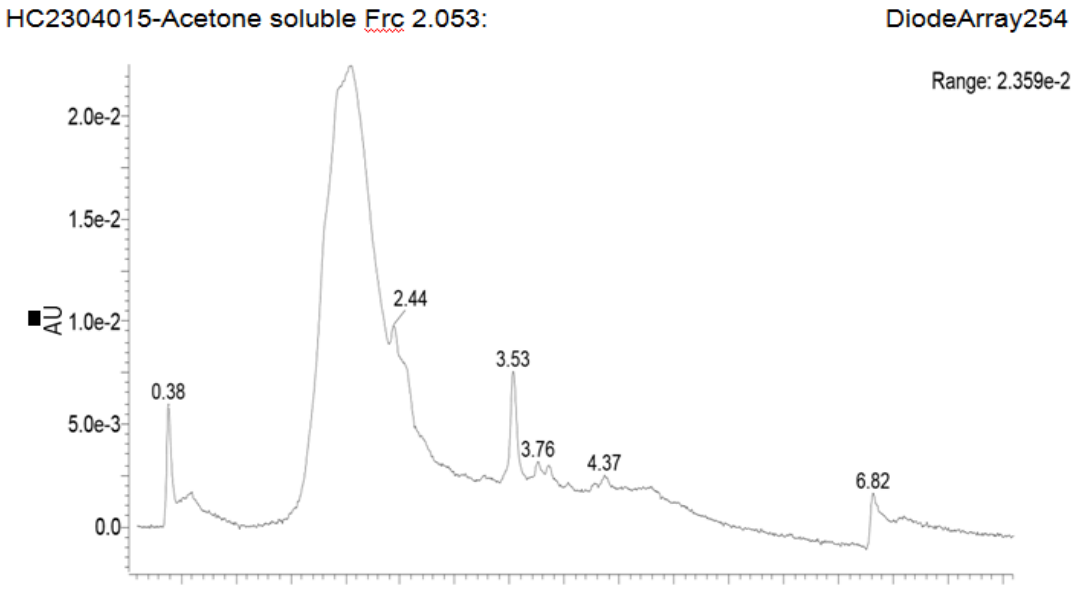

Figure 5 HPLC chromatogram of acetone-soluble fraction

Characterization and Identification of Isolated Compounds

Characterization of Isolated Compound DPE-1

Physical parameters of the compound

Physical state: White crystalline powder
Melting point: 211°C (lit.211-213°C)
Yield: 100 mg

The compound DPE-1gave a positive response for Liebermann-Burchard test for triterpenoids.

Spectral characteristics of DPE-1

FTIR (cm^{-1}): 3446.79 (OHstr.), 2929.87 (C-Hstr.in CH_3), 2866.22 (C-H str. in CH_2), 1639.49 (C=C str.), 1450.47 (C-H bend), 1377.17(gem dimethyl str.).

^{1}H NMR (DMSO) δ0.787(s,3H, H-23), δ 0.838 (s,3H,H-24), δ0.870(s,3H,H-25), δ0.912 (s,3H,H-26), δ0.939 (s,3H,H-27), δ0.987 (s,3H,H-28), δ1.004 (s,3H,H-30), δ1.235-1.623 {m,25H, (CH_2 and CH protons); H1, 2,5,6,7,9,11,12,13,15,16,18,19,21,22} δ 4.729 (d, 1H, H-29a); δ 4.678 (d,1H,H-29b) δ2.967 (s,1H,H-3).

^{13}C NMR (DMSO): δ38.240 (C-1); δ27.149(C-2); δ76.751 (C-3); δ38.87(C-4); δ54.812 (C-5); δ18.556 (C-6); δ33.852(C-7); δ40.13(C-8); δ49.786 (C-9); δ36.676 (C-10); δ20.26 (C-11); δ25.023 (C-12); δ38.49 (C-13); δ40.32 (C-14); δ28.083 (C-15); δ35.688 (C-16); δ42.104(C-17); δ48.315 (C18); 47.365 (C-19); δ150.210 (C-20); δ29.282 (C-21); δ39.918 (C-22); 28.282 (C-23); δ15.572 (C-24); δ15.925(C-25); δ15.808 (C-26); δ13.963 (C-27); δ17.925 (C-28); δ109.325(C-29); δ19.385(C-30)

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Mass Spectra

Molecular formula	$C_{30}H_{50}O$
Molecular weight	426 g/mol
ESI-MS (m/z)	427.3272(M+1) +The other peaks appeared at 411.3316, 393.3239, 369.2899, and 189.1540.

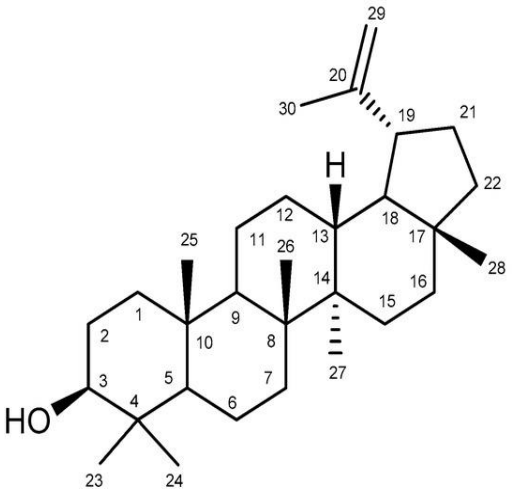
The FTIR spectrum of the compound exhibited abroad peak at 3446.79 cm^{-1} indicating the presence of a hydroxyl group. The peak at 2929.87cm^{-1} and 2866.22 cm^{-1} indicated the C- H stretching in CH_3 and CH_2 respectively. The peak at 1639.49 cm^{-1} indicated C=C stretching. The small peak at 1450.49 cm^{-1} indicated a C-H bend in germinal dimethyl. The peak at 1377.17cm^{-1} indicated gem dimethyl stretching.

The ^{1}H NMR spectrum of the compound exhibited singlet peaks at δ0.787,δ0.838,δ 0.870, δ0.912, δ0.939, δ0.987, and δ1.004 at H-23, 24, 25,26, 27, 28, 29, and 30, indicating. The tertiary methyl protons. The singlet at δ2.967 indicated a proton at H-3. The doublets at δ 4.729and δ 4.678are indicative of two olefinic protons at H-29aand H-29b.The multiplet between δ1.235 and 1.623 indicated methylene and methane protons.

The ^{13}C NMR spectrum exhibited the presence of 30 carbon atom signals for the pentacyclic triterpenoid of the lupine skeleton, which include seven methyl groups at δ28.282 (C-23), δ15.572(C-24), δ15.925(C-25), δ15.808 (C-26), δ13.963(C-27), δ17.925(C-28), δ19.385(C-30), eleven methylene, six methine groups and six quaternary carbon atoms. The signals at δ 150.210 (C-20) and 109.325 (C-29) were deshielded due to an olefinc bond betweenthem. Also,the signal at δ76.751(C-3) was deshielded due to the OH group at C-3.

The mass spectrum (ESI-MS) exhibited a molecular ion (M+1)+ peak at 427.3272 m/z corresponding to the molecular formula $C_{30}H_{50}O$. The other fragments appeared at m/z 411.3316, 393.3239, 369.2899, and 189.154.

From the melting point, FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectral data, the compound DPE-1 was identified as Lupeol.



lup-20(29)-en-3β-ol

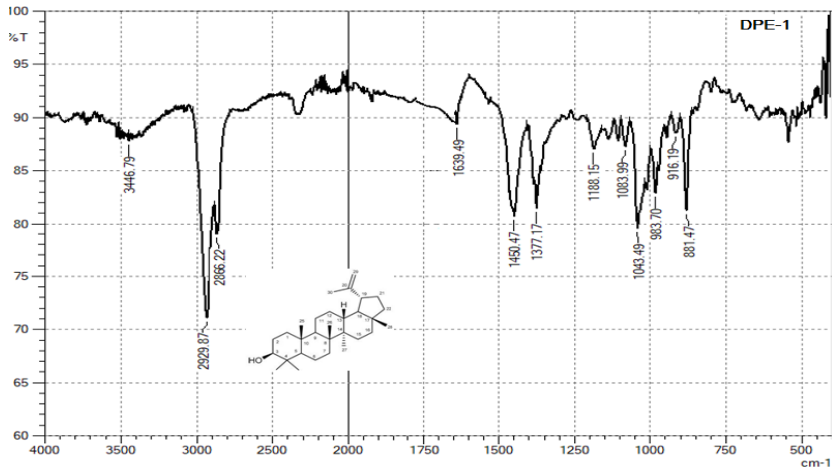


Figure 6 FTIR spectrum of compound DPE-1 (Lupeol)

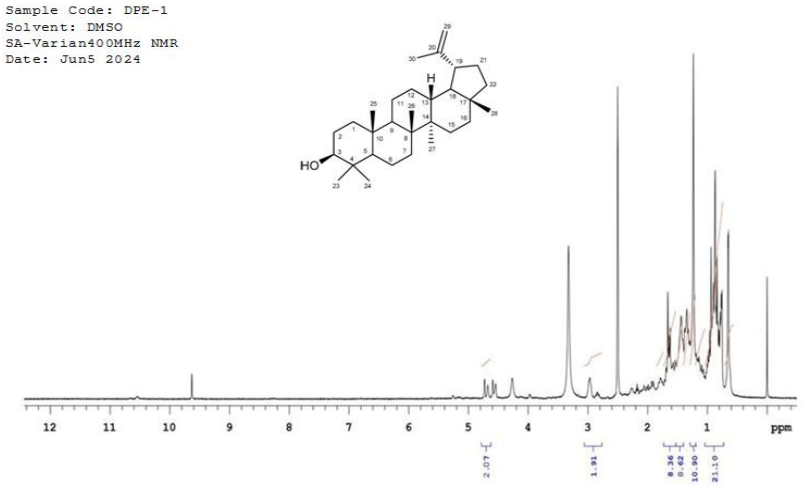


Figure 7 A ^{1}H NMR spectrum of compound DPE-1 (Lupeol)

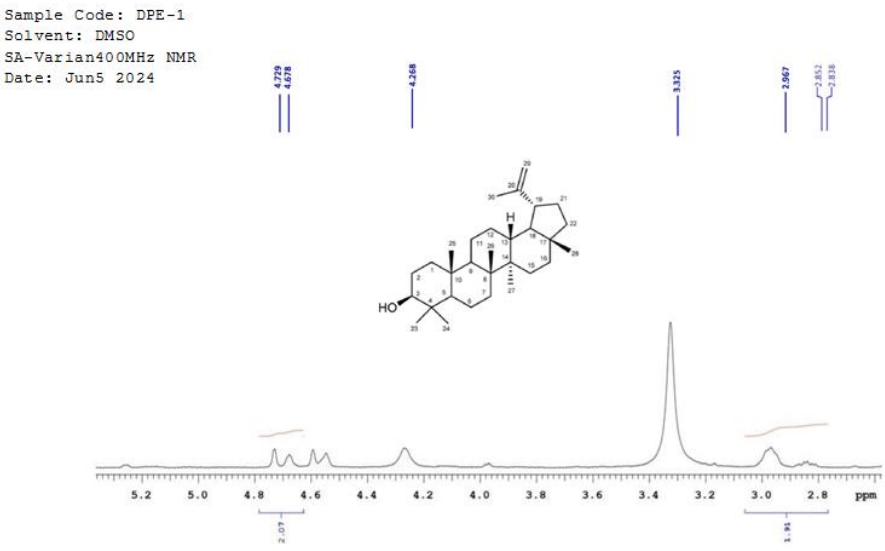


Figure 8 B ^{1}H NMR spectrum of compound DPE-1 (Lupeol)

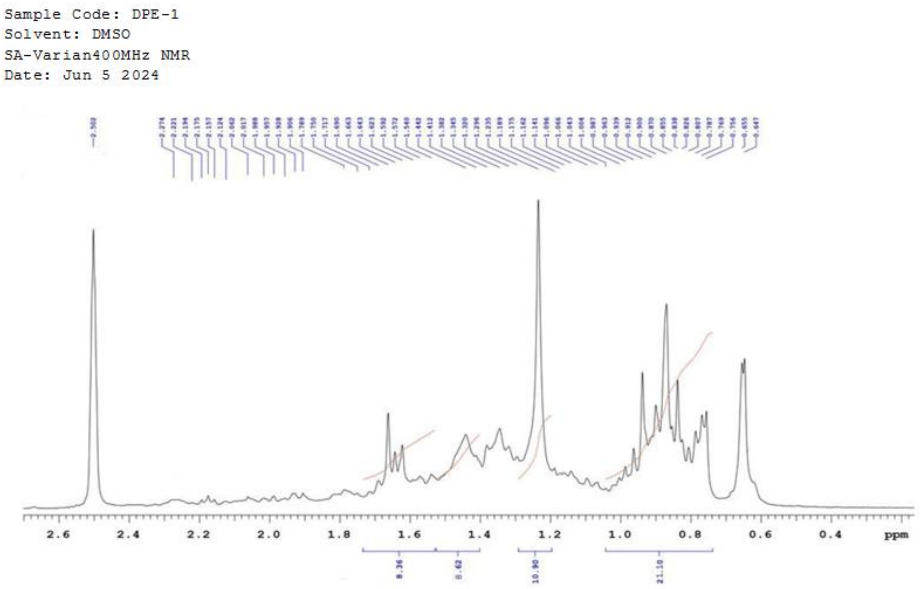


Figure 9 C [1]H NMR spectrum of compound DPE-1 (Lupeol)

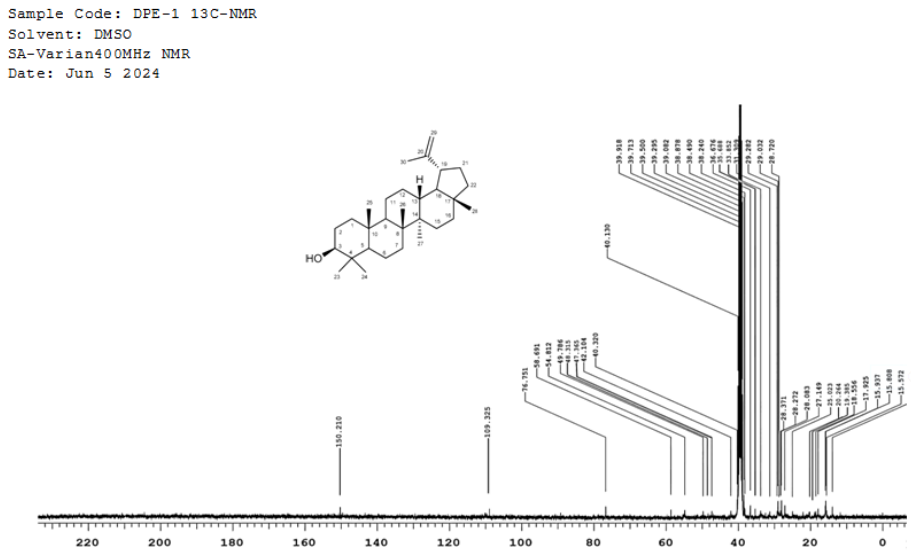


Figure 10 A [13]C NMR spectrum of compound DPE-1 (Lupeol)

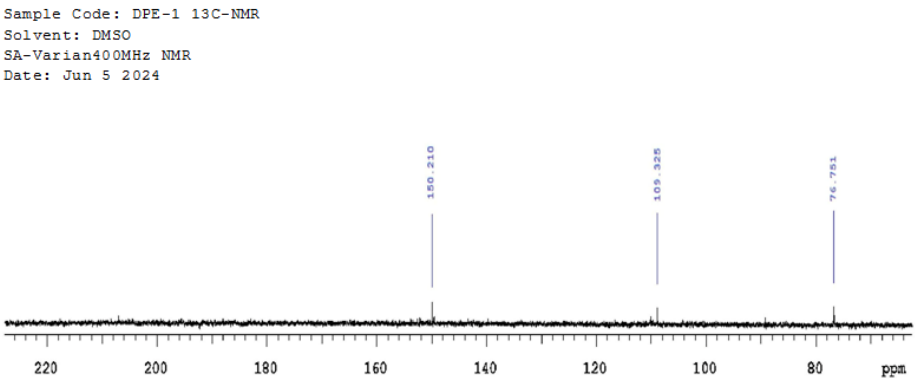


Figure 11 B [13]C NMR spectrum of compound DPE-1 (Lupeol)

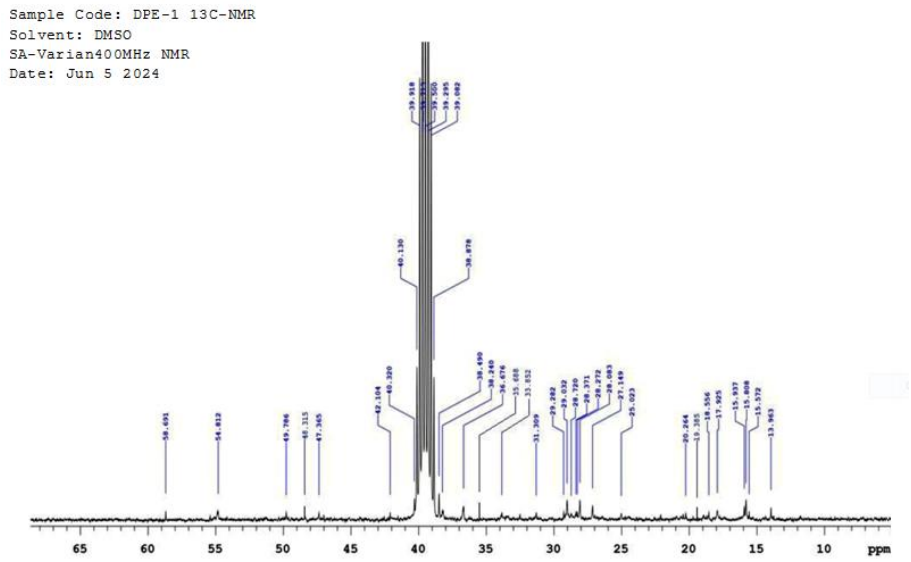


Figure 12 C ^{13}C NMR spectrum of compound DPE-1 (Lupeol)

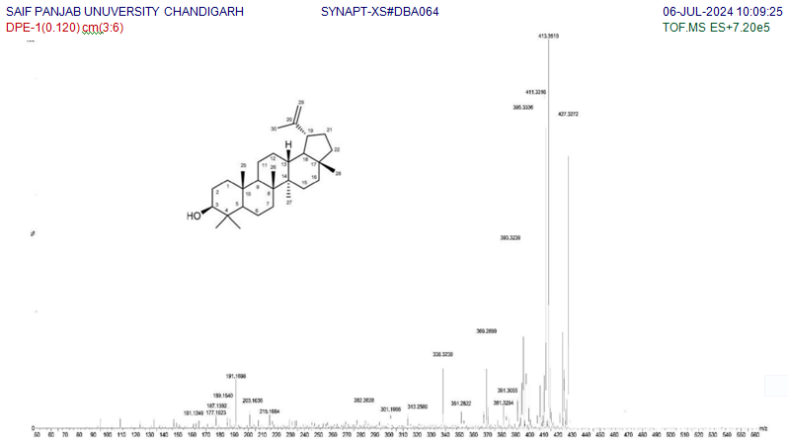


Figure 13 Mass spectrum of compound DPE-1 (Lupeol

Characterization of Isolated Compound DPE-2

Physical parameters of the compound

Physical state: White crystalline powder
Melting point: 255°C (lit.256-257°C)
Yield: 226 mg

The compound DPE-2 gave a positive response for Liebermann-Burchard test for triterpenoids.

Spectral characteristics of DPE-2

FTIR (cm^{-1}): 3444.87 (OH str.), 2929.87(C-H str in CH_3), 2866.22 (C-H str. in CH_2), 1687.71(C=C str.), 1452.40 (C-H deformation in germinaldimethyl), 1375.25 (gem dimethyl str.) and 1033.85(C-Ostr.in 2°alcohol).

^{1}H NMR (DMSO): δ0.648(s, 3H, H-23); δ 0.869 (s,3H,H-24); δ0.928 (s,3H,H-25); δ0.956 (s,3H,H-26) δ0.976 (s,3H,H-27); δ1.077(s,3H,H-30); δ1.410–2.417 {m,25H, (CH_2 and CH

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protons) H-1,2,5, 6,7,9,11,12,13,15,16,18,19,21,22} δ 4.559 (d, 1H, H-28a), δ553 (d,1H,H-28b). δ4.685 (d,1H,H-29a), δ4.660(d,1H,H-2b). δ3.968 (s,1H, H-3).

^{13}C NMR (DMSO): δ39.08 (C1); δ18.73 (C-2); δ76.76 (C-3); δ39.5 (C-4); δ54.86 (C-5); δ17.95 (C-6); δ36.63 (C-7); δ40.43 (C-8); δ49.90 (C-9); δ38.22 (C-10) ;δ24.80 (C-11);δ26.6 (C-12); δ37.57 (C-13); δ42.18 (C-14); δ27.14(C-15); δ29.17 (C-16); δ47.29 (C-17); δ47.37 (C-18); δ48.50 (C-19); δ150.29; (C-20);δ29.00 (C-21); δ33.79 (C-22); δ28.07 (C-23); δ 15.70 (C-24); δ15.93 (C-25) ;δ15.95 (C-26); δ14.49 (C-27); δ55.39 (C-28); δ109.61(C-29); δ20.33(C-30).

Mass Spectra:

Molecular formula	$C_{30}H_{50}O_2$
Molecular weight	442 g/mol
ESI-MS(m/z):	443.3269(m+1)+.The other peaks appeared at 439.3308,425.3468,411.3316,407.3361,395.3408,217.1814, and 189.1540.

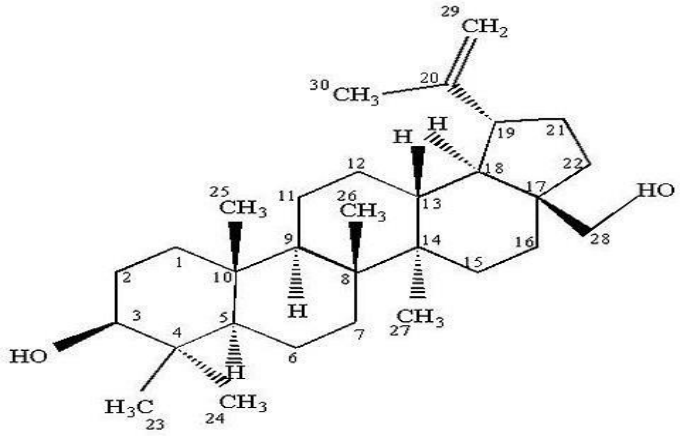
The compound DPE-2 was obtained as a white crystalline powder, having a melting point of 255°C.It gave a positive response for Liebermann-Burchard test for triterpenoids.

The FTIR spectrum of the compound exhibited a broad peak at 3444.87 cm^{-1} indicating the presence of ahydroxyl group. The peaks at 2929.87 cm^{-1} and 2866.22 cm^{-1} indicated the C-H stretching in CH_3 and CH_2, respectively. The peak at 1687.71 cm^{-1} indicates C=C stretching. The small peak at 1452.40cm^{-1} indicated a C-H bend in germinal dimethyl. The peak at1375.25 cm^{-1} indicated gem dimethyl stretching. The peak at1033.85cm^{-1} indicated the C-O str. in secondary alcohol.

The ^{1}H NMR spectrum of the compound exhibited the singlets at δ 0.648,δ 0.869,δ 0.928, δ 0.956, δ 0.976, and δ 1.077 at H-23, 24, 25, 26, 27, and 30, indicating the tertiary methyl protons. The doublet at δ3.968 indicated proton at H-3.The doublet at δ4.559 and δ4.553 indicated protons at H-28 and H-28b, while δ4.685 (H-29a),and δ4.660(H-29b) are indicative of two olefinic protons. The multiplet, δ 1.410 -2.417, indicated methylene and methineprotons.

The ^{13}C NMR spectrum exhibited the presence of 30 carbon atom signals for the pentacyclic triterpenoid of the lupane skeleton, which includes six methyl groups at δ28.07 (C-23), δ15.70 (C-24), δ15.93 (C-25), δ15.95 (C-26), δ14.49 (C-27), δ20.33 (C-30); twelve methylene, six methane and six quaternary carbon atoms. The signals at δ150.29 (C-20) and 109.61 (C-29), were deshielded due to an olefinic bond between them. Also, signal at δ76.751 (C-3) and δ 55.39 (C-28) was deshielded due to the OH group at C-3 and C-28.

The mass spectrum (ESI-MS) exhibited a molecular ion (M+1)+ peak at 443.3269m/z corresponding to the molecular formula $C_{30}H_{50}O_2$.The other fragments appeared at m/z 439.3308, 425.3468, 411.3316, 407.3361, 395.3408, 217.1814, and 189.1540. From the melting point, FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectral data, the compound DPE-2 was identified as Betulin.



3-lup-20(29)-ene-3β, 28-diol

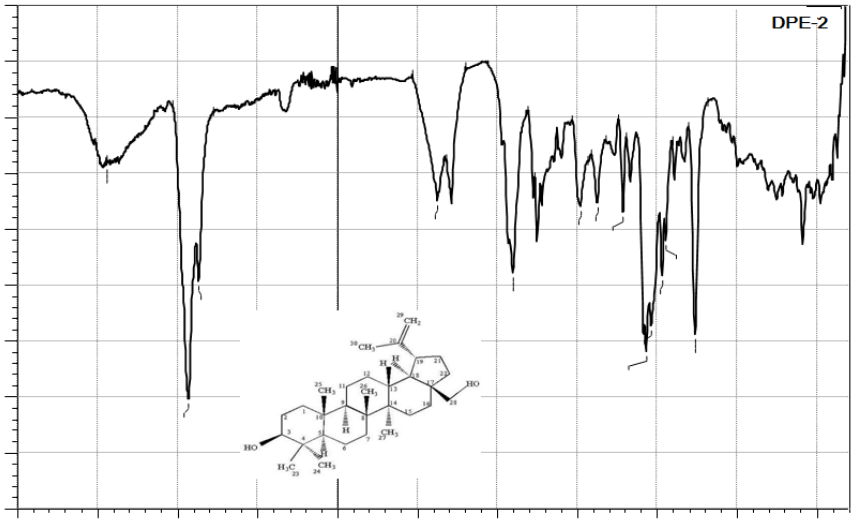


Figure 14 FTIR Spectrum of compound DPE-2 (Betulin)

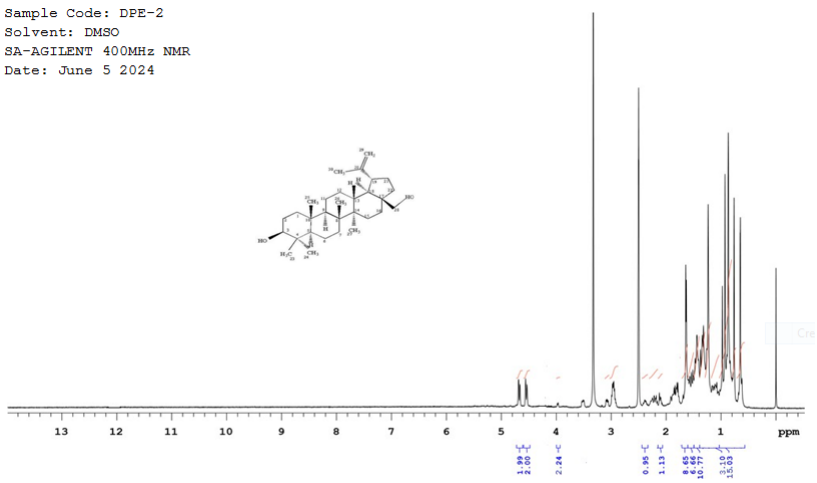


Figure 15 A [1]H NMR Spectrum of compound DPE-2 (Betulin)

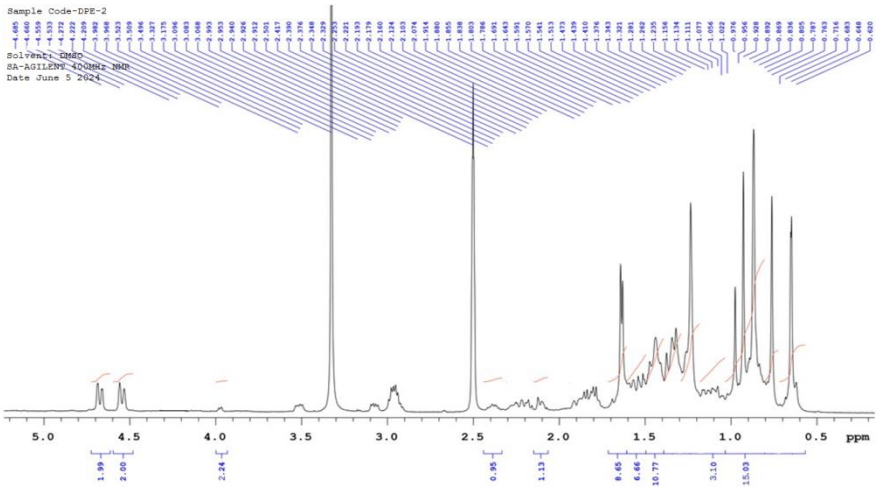


Figure 16 B ^{1}H NMR spectrum of compound DPE-2 (Betulin)

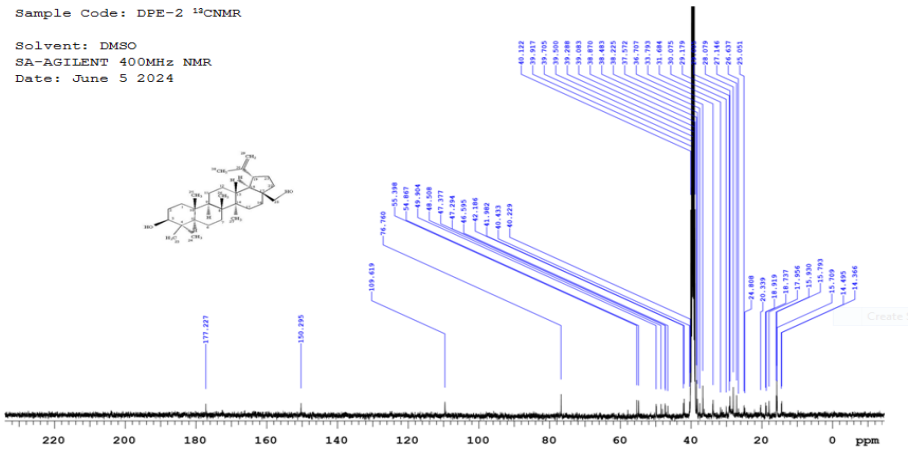


Figure 17 A ^{13}C NMR spectrum of compound DPE-2 (Betulin)

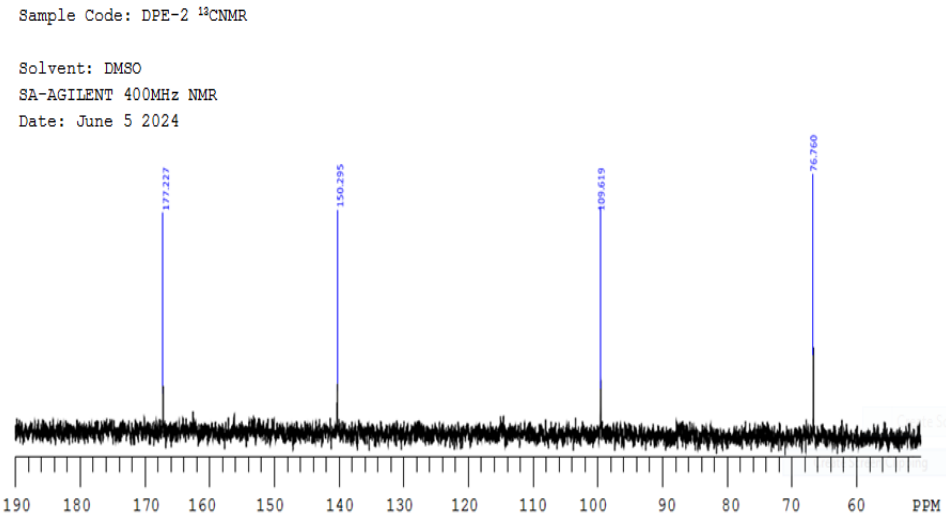


Figure 18 B ^{13}C NMR spectrum of compound DPE-2 (Betulin)

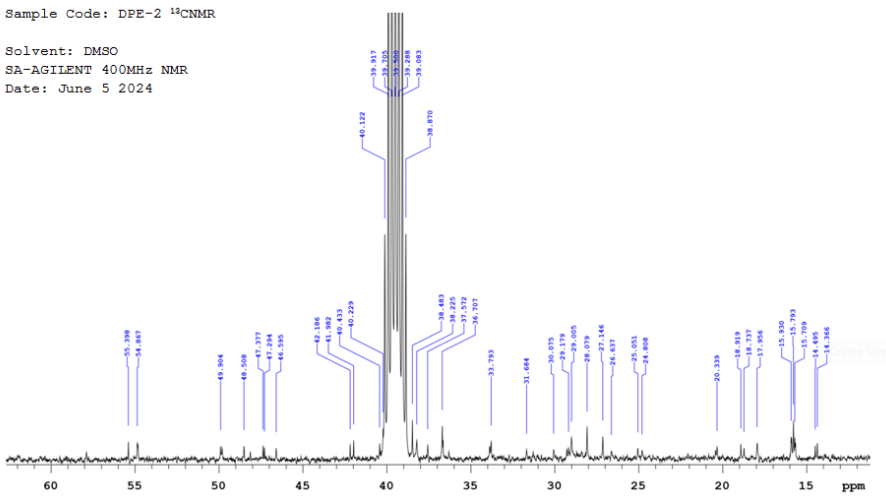


Figure 19 C ^{13}C NMR spectrum of compound DPE-2 (Betulin)

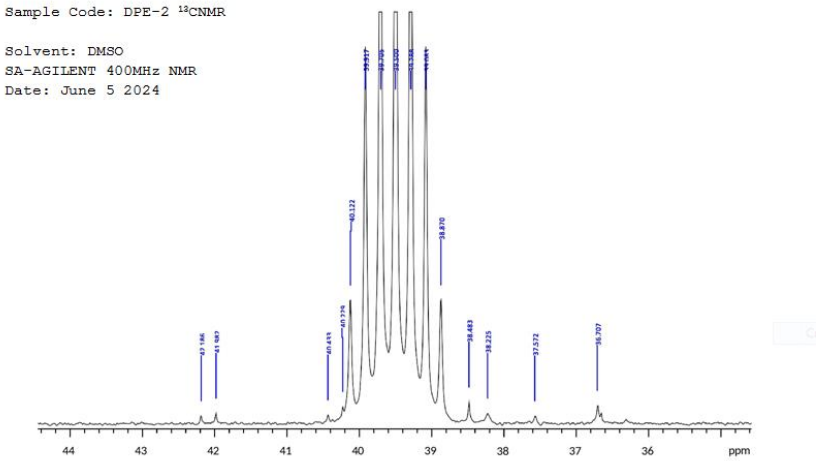


Figure 20 D ^{13}C NMR spectrum of compound DPE-2 (Betulin)

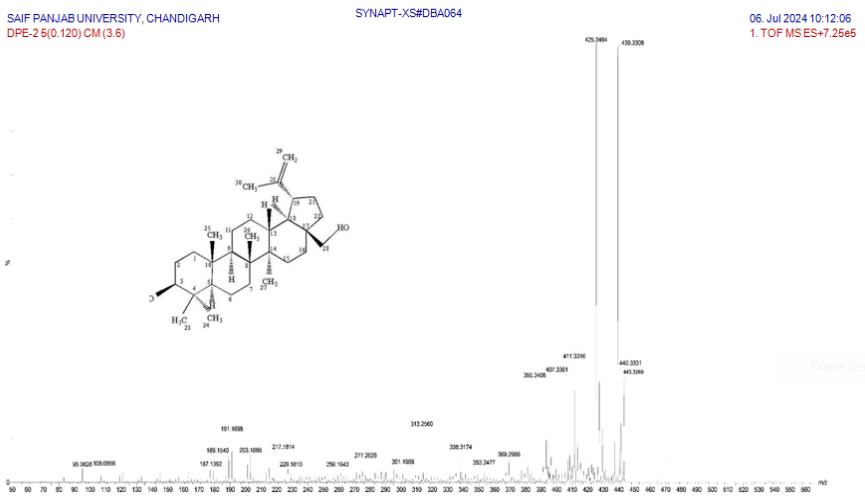


Figure 21 Mass Spectrum of compound DPE-2 (Betulin)

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Characterization of Isolated Compound DPE-3

Physical parameters of the compound

Physical state: Yellowish white crystals
Melting point: 283°C (lit.283-285°C)
Yield: 662 mg

The compound DPE-3 gave a positive response for Liebermann-Burchard test for triterpenoids.

Spectral characteristics of DPE-3

FTIR (cm^{-1}): 3442.94 (OH str.), 2937.59 (C-H str. in CH_3), 2870.08 (C-H str. in CH_2), 1685.79 (C=O), 1641.42 (C=C str.), 1450.47 (C-H deformation in germinaldimethyl), 1377.17 (gem dimethylstr.) and 1043.49 (C-Ostr.in 2°alcohol).

^{1}H NMR (DMSO): δ0.867 (s,3H,H-23); δ0.648 (s,3H,H-24); δ0.762 (s,3H,H-25); δ0.929 (s,3H,H-26); δ0.830(s,3H,H-27); δ1.098 (s,3H,H-30); δ1.126-.953{m,25H, (CH_2 and CH protons), H-1,2,5,6,7,11,12,13,15,16,18,19,21,22}; δ 4.559 (s, 1H, H-29a); δ4.685 (s,1H,H-29b); δ 4.259 (d,1H,H-3); δ12.052 (s,1H,COOH)

^{13}C NMR (DMSO): δ39.08 (C-1); δ27.14 (C-2); δ76.74 (C-3); δ39.91 (C-4); 55.39 (C-5); δ17.94 (C-6); δ33.88 (C-7); δ40.13 (C-8); δ49.89 (C-9); δ38.47 (C-10); δ20.43 (C-11); δ25.05 (C-12); δ38.78 (C-13); δ41.97 (C-17); δ29.16 (C-15); δ31.68 (C-16); δ54.85 (C-17); δ46.59 (C-18); δ49.89(C-19); δ150.31 (C-20); δ30.07(C-21); δ38.22(C-22); δ28.07(C-23); δ15.70(C-24); δ15.78 (C-25); δ15.92 (C-26); δ14.35 (C-27); δ177.22 (C-8); δ114.85 (C-29); δ18.92 (C-30).

Mass Spectra

Molecular formula:	$C_{30}H_{48}O_3$
Molecular weight:	456 g/mol
ESI-MS (m/z):	457.3285(m+1)+.The other peaks appeared at 439.3306, 425.3464, 411.3316, 203.1686, and 189.1540

The compound DPE-3 was obtained as yellowish white crystals, having a melting Point of 283°C.It gave a positive response for Liebermann-Burchard test for triterpenoids.
The FTIR spectrum of the compound exhibited a broad peak at 3442.94cm^{-1}, indicating the presence of a hydroxyl group. The peaks at 2937.59 cm^{-1} and 2870.08 cm^{-1} indicated the C-H stretching in CH_3 and CH_2, respectively. The prominent peak at 1685.79 cm^{-1} indicated the carboxylic group. The peak at 1641.42 cm^{-1} indicated C=C stretching. The small peak at 1450.47cm^{-1} indicated a C-H bending of germinal dimethyl. The peak at 1377.17cm^{-1} indicated gemdimethyl stretching. The peak at 1043.49cm^{-1} indicated the C-O str. in secondary alcohol.

The ^{1}H NMR spectrum of the compound exhibited the singlet peaks at δ0.867,δ0.648, δ0.762, δ 0.929,δ 0.830, and δ 1.098 at H-23, 24,25, 26, 27, and 30, indicating the tertiary methyl protons. The singlets at δ 4.259 and δ 12.052 indicated protons at H-3 and COOH, respectively. The singlets at δ4.559 (H-29a) and δ4.685 (H-29b) are indicative of olefinic protons. The multiplet δ1.126-2.953 indicated methylene and methane protons.

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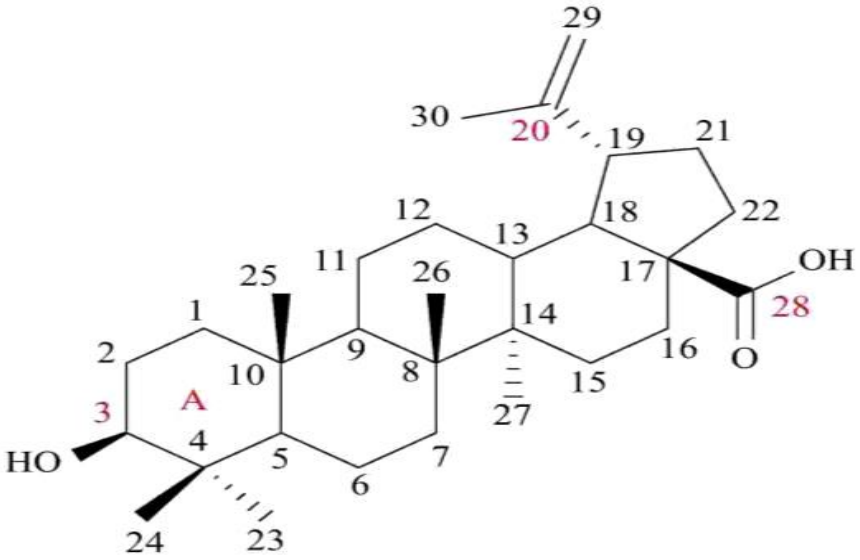
The ^{13}C NMR spectrum exhibited the presence of 30 carbon atom signals for the pentacyclic triterpenoid of the lupane skeleton, which include six methyl groups at 28.07(C-23), 15.70(C-24), 15.78(C-25), 15.92(C-26), 14.35 (C-27), 177.22 (C-8), 144.85 (C-29), 18.92 (C-30); eleven methylene, six methine, six quaternary and one carbonyl carbon atoms. The signals at δ 150.31 (C-20) and 109.61 (C-29) were deshielded due to an olefinic bond between them. Also signals at δ76.74 and δ177.22 were deshielded due to the OH and COOH groups at C-3 and C-28, respectively.

The mass spectrum (ESI-MS) exhibited a molecular ion (M+1)+peak at 457.3285m/z corresponding to the molecular formula $C_{30}H_{48}O_3$.The other fragments appeared at m/z 439.3306, 425.3464, 411.3316, 203.1686, and 189.1540. From the physical and spectral data, the compound DPE-3 was identified as Betulinic acid.



3β-hydroxy-lup-20(29)-en-28-oic acid

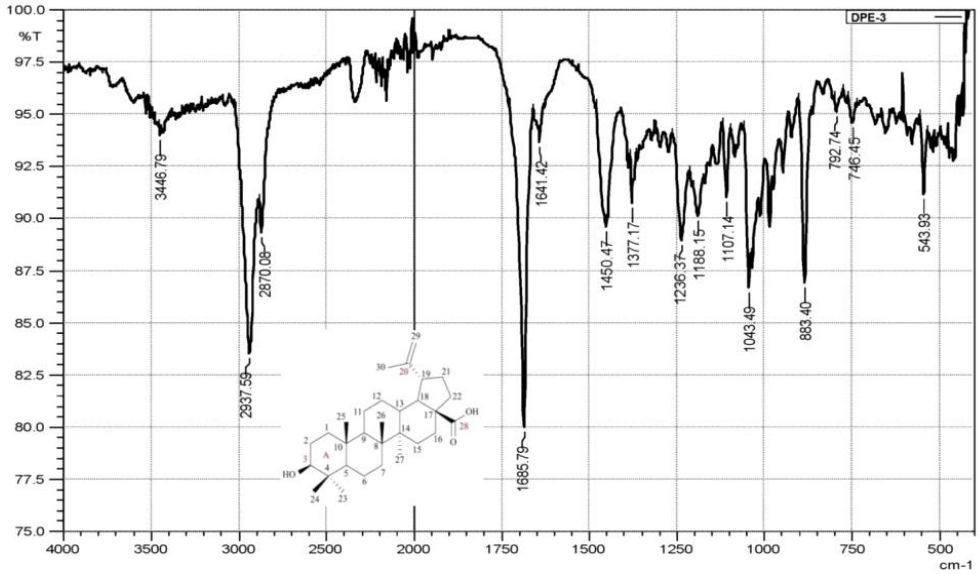


Figure 22 FTIR spectrum of compound DPE-3 (Betulinic acid)

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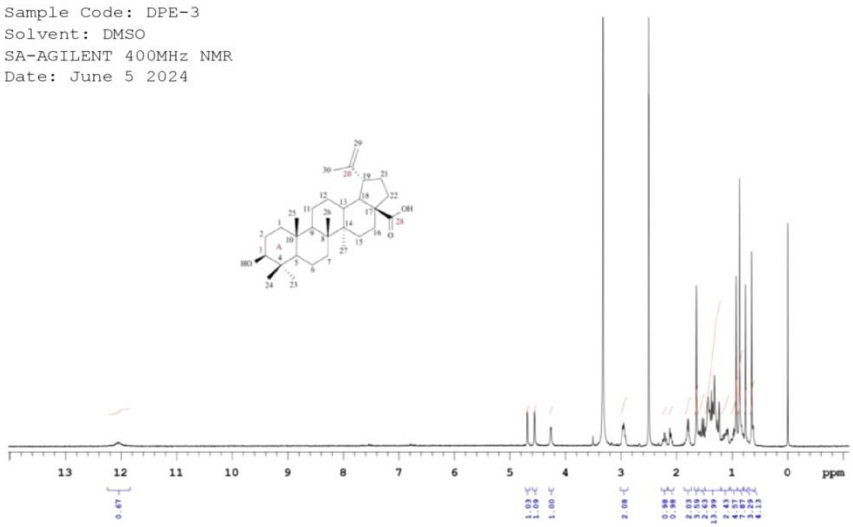

Figure 23 A ^{1}H NMR Spectrum of Compound DPE-3 (Betulinic acid)

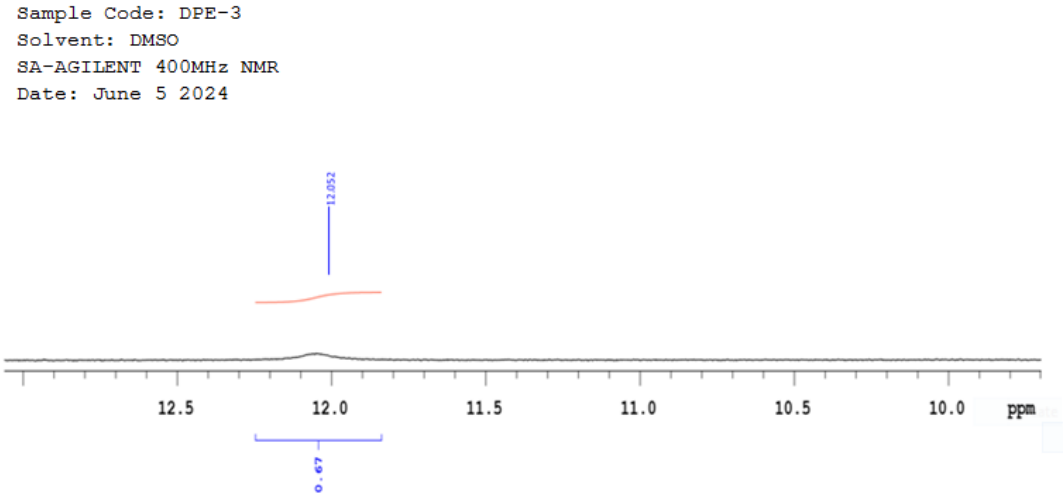

Figure 24 B ^{1}H NMR Spectrum of Compound DPE-3 (Betulinic acid)

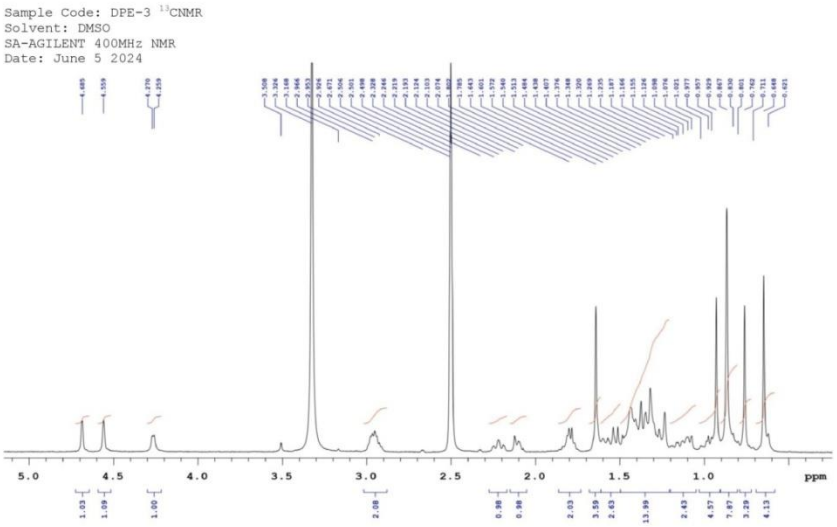

Figure 25 A ^{13} C NMR Spectrum of Compound DPE-3 (Betulinic acid)

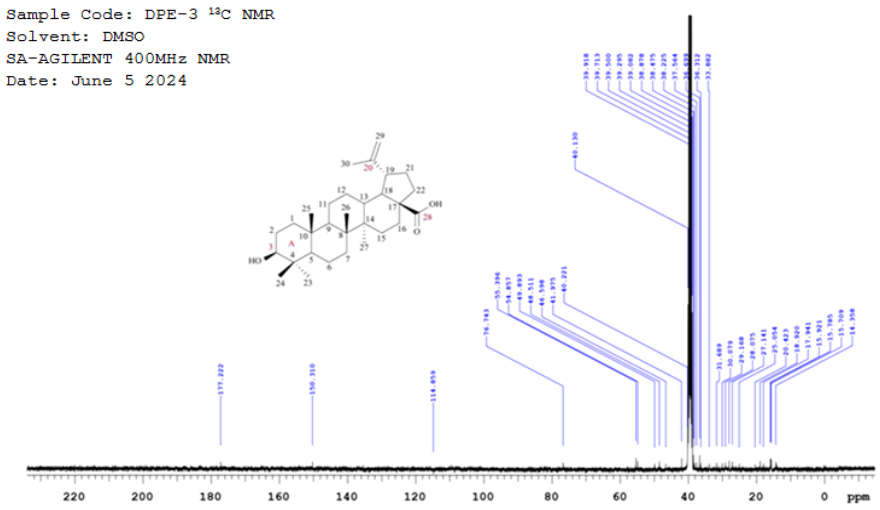

Figure 26 B [13] C NMR Spectrum of Compound DPE-3 (Betulinic acid)

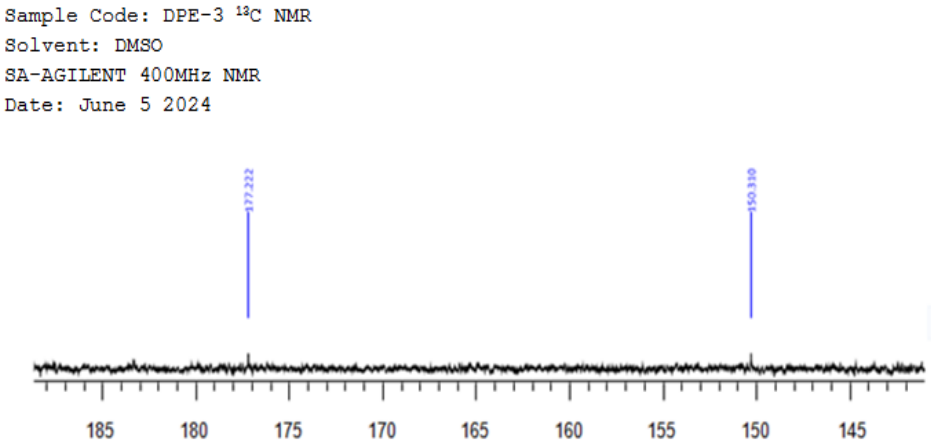

Figure 27 C [13] C NMR Spectrum of Compound DPE-3 (Betulinic acid)

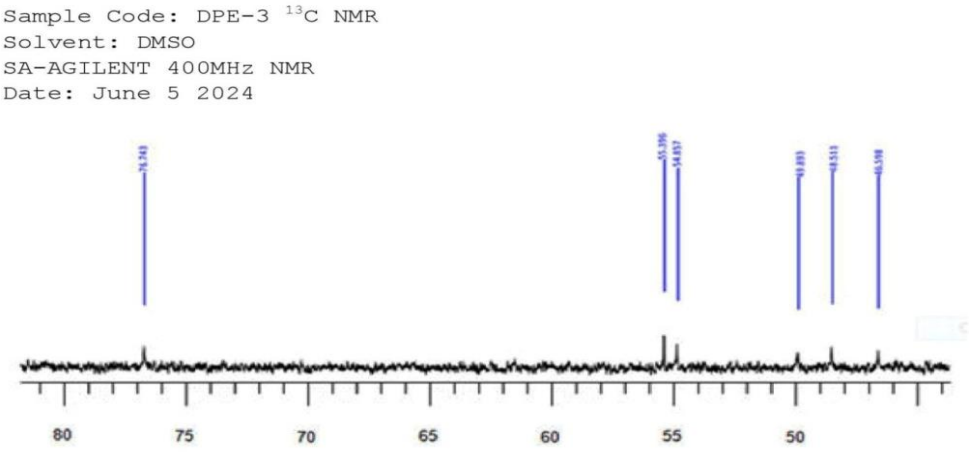

Figure 28 D [13] C NMR Spectrum of Compound DPE-3 (Betulinic acid)

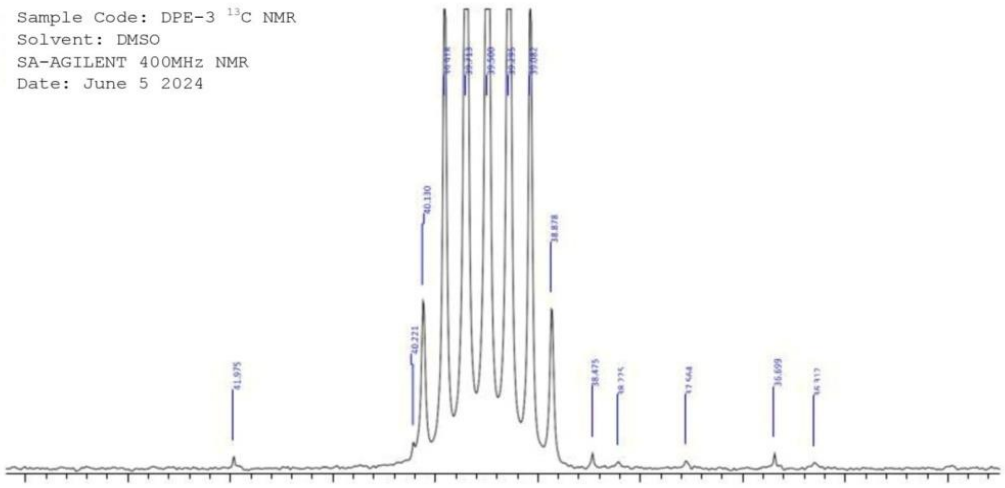

Figure 29 E [13] C NMR Spectrum of Compound DPE-3 (Betulinic acid)

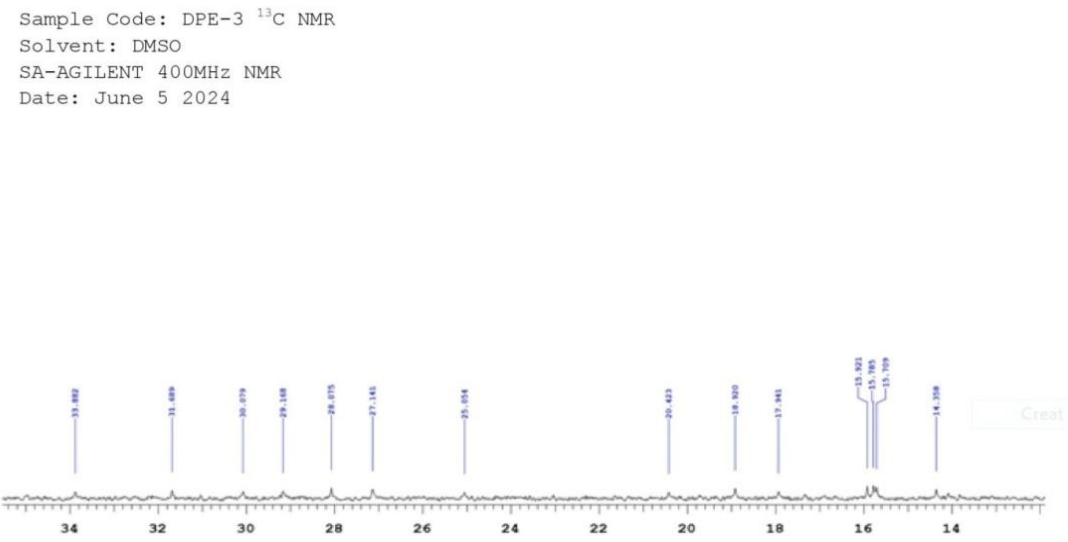

Figure 30 F [13]C NMR Spectrum of Compound DPE-3 (Betulinic acid)

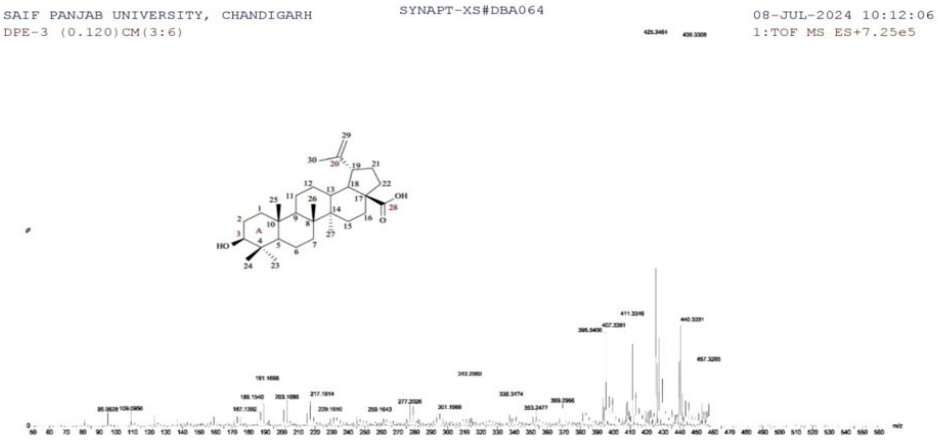

Figure 31 Mass Spectrum of Compound DPE-3 (Betulinic acid)

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Characterization of Isolated Compound DPE-A1

Physical parameters of the compound

Physical state: White crystalline powder
Melting point: 134°C (lit.134-138°C)
Yield: 100mg

The compound DPE-A1 gave a positive response for the Liebermann-Burchard test for triterpenoids.

Spectral characteristics of DPE-A1

FTIR (cm^{-1}): 3416.44 (OH str.), 2957.97, 2937.44 (C-H str. In CH_3), 2868.10 (C-H str. in CH_2), 1738.43, 1637.44, 1639.49 (C=Cstr.), 1462.80 (C-H deformation).

^{1}H NMR (DMSO): δ0.6542-δ0.6740 (m,3H,CH3,C-29); δ0.7817-δ0.8284 (m, 6H, CH_3, C-26, C-27); δ0.9020-δ1.0696 (m,9H,CH_3, C-18,C-19,C-21); δ1.1150-δ1.6571(m,22H,CH_2,C-1,C-2,C-4,C-7,C-11,C-12,C-15,C-16,C-22,C-23,C-28); δ1. 7401-δ1.7740 (d,2H,CH,C-24,C-25); δ1.9134 (s,2H,CH,C-17,C-20); δ 2.0672- δ 2.1893 (m,4H, CH,C-3,C-8,C-9,C-14) δ 5.0398- δ 5.1555 (d, 1H, OH); δ5.2671-δ5.6253(d, 1H,C-6)

^{13}C NMR (DMSO): δ35.96 (C-1); δ23.76 (C-2); δ78.59 (C-3); δ45.04 (C-4); δ162.90(C-5); δ120.30 (C-6); δ22.50 (C-7); δ28.60 (C-8); δ47.58 (C-9); δ31.31 (C-10); δ19.61(C-11); δ36.83 (C-12); δ42.11 (C-13); δ69.90 (C-14); δ20.83 (C-15); δ20.93 (C-16); δ49.52 (C-17); δ15.17 (C-18); δ 18.83 (C-19); δ41.75 (C-20); δ20.51 (C-21); δ28.91 (C-22); δ18.74 (C-23); δ47.41 (C-24); δ19.93 (C-25); δ18.51(C-26); δ18.04(C-27); δ19.03(C-28); δ11.57-12.01(C-29).

Mass Spectra

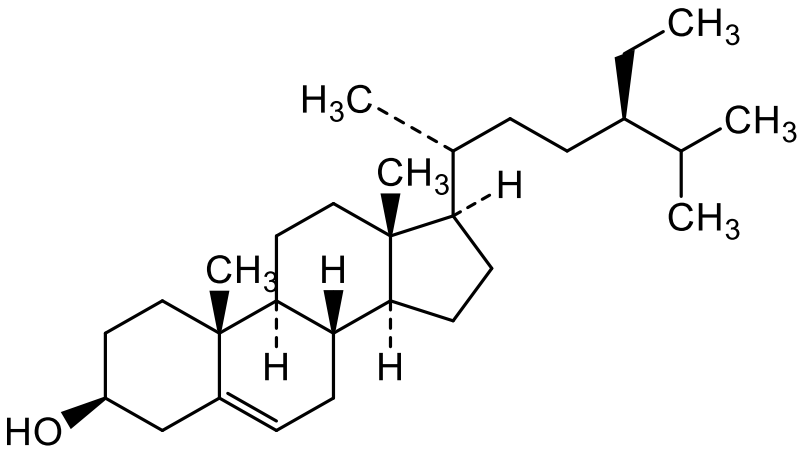
Molecular formula: $C_{29}H_{50}O$

Molecular weight: 414.3832g/mol

ESI-MS (m/z) : 415.2123(M+1)+. The other peaks were obtained at m/z 410, 396, 344, 342, 293, 255, 209, 167, and 149.

The compound DPE-A1 was obtained as a pearl white powder, having a melting point of 134°C.Itgave a positive response for the Liebermann-Burchard test for triterpenoids. The FTIR spectra of the compound exhibited a broad peak at 3414.44cm^{-1} which indicated the presence of hydroxyl group. The absorption peaks obtained at 2957.97cm^{-1} and 2937.44 cm^{-1} indicate the C-H str. in CH_3. Peaks at 2868.10 cm^{-1} corresponds to C-H str. in CH_2,1738.43cm^{-1},1637.44cm^{-1} and1617.34cm^{-1} corresponds to C=C str. The peak obtained at 1462.80 cm^{-1} corresponds to C-H deformation. The ^{1}H NMR spectra exhibited multiplet in the range of δ0.6542-δ1.0696 that correspond to the six terminal methyl groups at C-18, C-19, C-21, C-26, C-27 and C-29. The peaks at δ 1.1150- δ1.6571 exhibited a multiplet accounting for the 22 methylene protons at C-1,C-2,C-4,C-7,C-11,C-12,C-15,C-16,C-22,C-23 andC-28.The peaks at δ1.7401- δ 2.1893 corresponds to the methyine protons at C-3,C-8,C-9,C-14,C-17,C- 20,C-24and C-25.The signals at δ5.2671-δ 5.6253corresponds to the vinylic proton at C-6. The ^{13}C NMR spectra shows signals at δ162.90 and δ120.30 that corresponds to the vinylic carbons at C-5and C-6. The oxygenated carbon at C-3s hows signals at δ 79.12- δ 78.59. The mass spectrum (ESI-MS) showed molecular ion peak at 414.3832 m/z corresponding to the molecular formula $C_{29}H_{50}O$. The peak at m/z 415.2123 corresponds to the (M+1)+ peak. Dehydration of the molecular ion peak resulted in a peak at

m/z 396.The cleavage of the side chain C_{17}-C_{20}, followed by dehydration resulted in a peak at m/z255. From the melting point, FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectral data, the compound DPE-A1 was identified as β- sitosterol.



5-Stigmasten-3β-ol

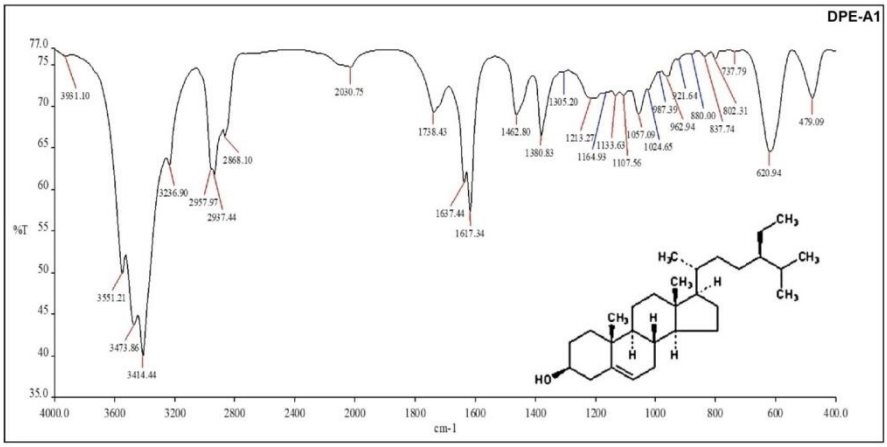


Figure 32 FTIR Spectrum of Compound DPE-A1 (β-Sitosterol)

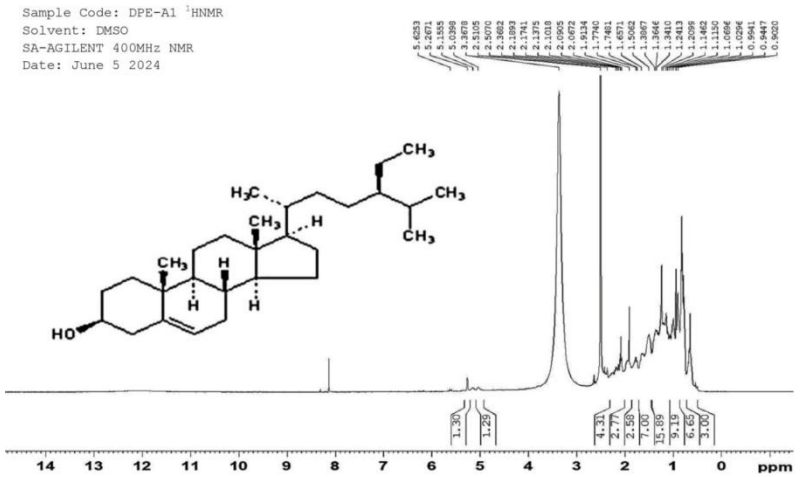


Figure 33 ^{1}H NMR Spectrum of Compound DPE-A1 (β-Sitosterol)

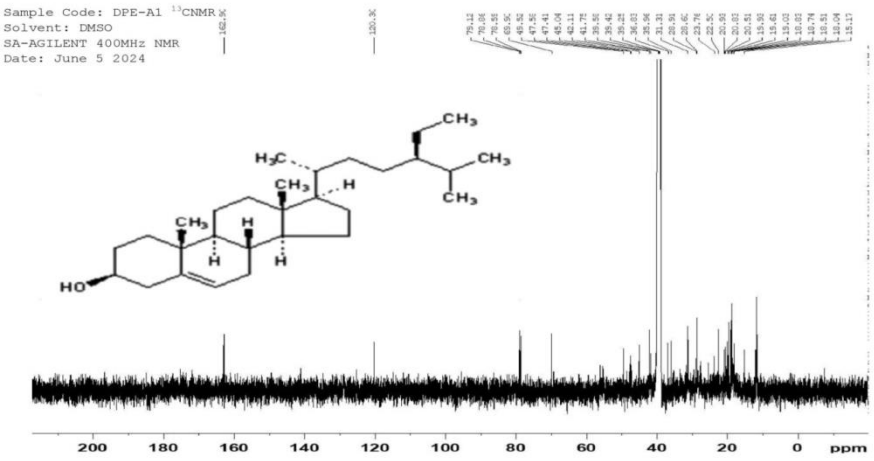


Figure 34 A ^{13}C NMR Spectrum of Compound DPE-A1 (β-Sitosterol)

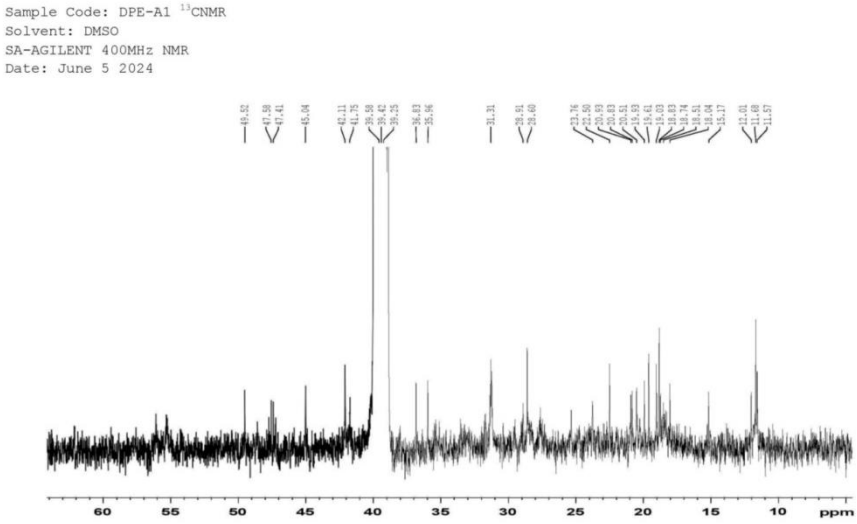


Figure 35 B ^{13}C NMR Spectrum of Compound DPE-A1 (β-Sitosterol)

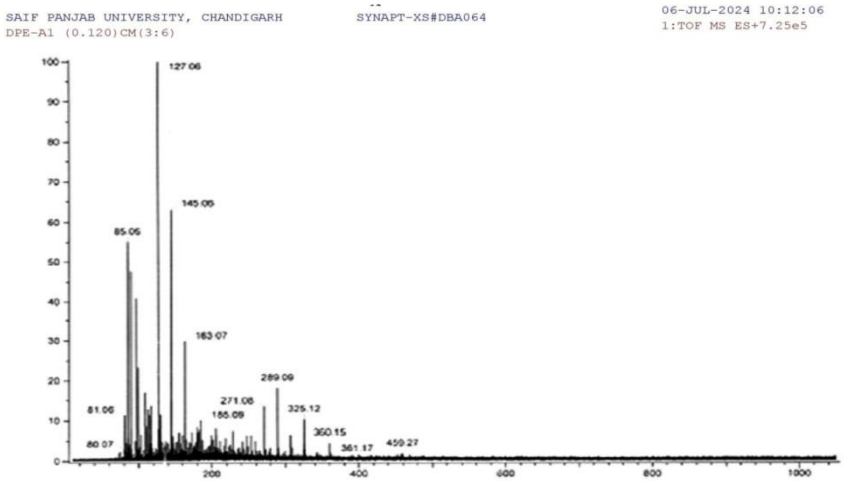


Figure 36 Mass Spectrum of Compound DPE-A1 (β-Sitosterol)

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***In Vitro* Anti Cancer Activity**
Cell line: MCF7 (Breast Cancer Cells)
Media: DMEM with high glucose (Cat No-11965-092), FBS (Gibco, In vitro gen) Cat No-10270106.
Antibiotic – Antimycotic 100X solution (Thermo fisher Scientific)-Cat No-15240062.

Table 7.4 Effects of compound against MCF7 Celllines

SR NO	SAMPLE CODE	Conc. (µg/ml)	OD			Mean	% of Inhibition	% of Viability	IC50 (µg/ml)
1	Control		1.534			-	-	-	-
2	Standard	6.25	0.977	0.975	0.979	0.977	36.31%	63.69%	
	5,Flurour-acil	12.5	0.746	0.744	0.748	0.746	51.36%	48.64%	
		25	0.519	0.521	0.518	0.519	66.16%	33.84%	
		50	0.389	0.389	0.387	0.388	74.70%	25.3%	38.12
		100	0.246	0.248	0.244	0.246	83.96%	16.04%	
3	Extract Syrup	6.25	1.307	1.305	1.308	1.306	14.86%	85.14%	
		12.5	1.058	1.056	1.055	1.056	31.16%	68.84%	
		25	0.911	0.913	0.909	0.911	40.61%	59.39%	61.25
		50	0.708	0.711	0.709	0.709	53.78%	46.22%	
		100	0.518	0.516	0.521	0.518	66.23%	33.77%	

**NE- Not Evaluable*

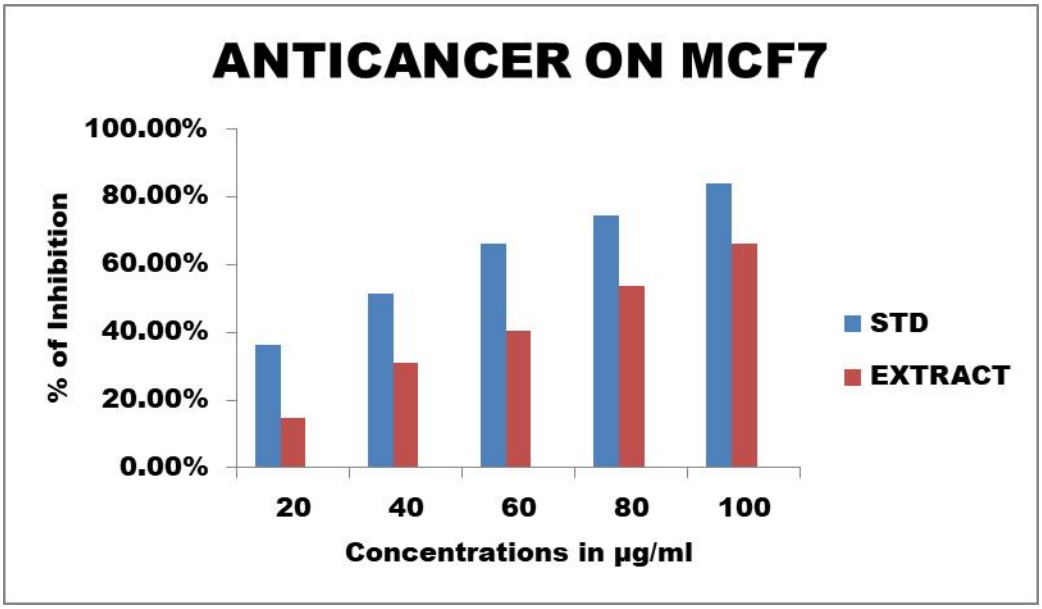

Figure 7.37: Effects of STD compound against extract

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eISSN No.: 2582-8371

At the different Concentrations extract shows the high percentage of inhibition against breast cancer cell line as compared to standard drug.

CONCLUSION

The present study successfully unveiled the phytochemical and anticancer potential of *Dillenia pentagyna Roxb.* bark extract. The ethanolic extract displayed rich secondary metabolite content, particularly flavonoids and triterpenoids. Quantitative estimations confirmed substantial phenolic (62.20 mg GAE/g) and flavonoid (58.26 mg QUE/g) concentrations. Chromatographic separation followed by spectral characterization led to the identification of key triterpenoids such as lupeol, betulin, betulinic acid, and β-sitosterol. In-vitro cytotoxic studies against MCF-7 breast cancer cell lines revealed potent anticancer activity, with DPE-2 and DPE-3 exhibiting significant inhibition and favorable IC_{50} values. The outcome suggests that these naturally derived compounds hold promise as lead molecules for anticancer drug development. This research supports the traditional therapeutic use of *D. pentagyna* and adds scientific value through modern pharmacological and spectral evidence. Further in-vivo studies and mechanistic evaluations are warranted to validate and optimize their efficacy and safety. The integration of phytochemical profiling and biological evaluation emphasizes the importance of this plant in natural product-based drug discovery.

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eISSN No.: 2582-8371

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