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Phytochemical Exploration of *Stevia rebaudiana*: Spectroscopic Characterization of Natural Constituents and Novel Semisynthetic Derivatives (LUM1-LUM7)

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ABSTRACT

This study successfully isolated stevioside from *Stevia rebaudiana* leaves via optimized solvent extraction (aqueous, acidic, basic, and alcoholic), followed by purification through rotary evaporation and recrystallization. The isolated stevioside served as a precursor for the synthesis of seven novel semisynthetic derivatives (LUM1–LUM7), chemically modified to introduce diverse functional groups including amino, imino, formyl, carboxylic acid, carbamoyl, anhydride, and ethyl ester moieties. Comprehensive structural elucidation was achieved using Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (^{1}H and ^{13}C NMR), and mass spectrometry (MS). Spectral analysis confirmed the successful modifications, with key signatures such as ester carbonyl stretches (1720–1750 cm ^{1} in FTIR), anomeric proton signals (δ 4.5–6.0 ppm in ^{1}H-NMR), and precise molecular ion peaks (e.g., *m/z* 805.3421 for stevioside and *m/z* 891.3940 for the anhydride derivative LUM6). The generation of this structurally diverse library of steviol glycoside derivatives demonstrates the potential of stevioside as a versatile scaffold for semisynthetic innovation. This work provides a foundational framework for exploring structure-activity relationships, paving the way for the development of enhanced sweeteners and novel bioactive compounds with potential applications in food science, nutraceuticals, and pharmaceutical industries.

Keywords: *Stevia rebaudiana*, steviol glycosides, semisynthetic derivatives, solvent extraction, spectral analysis, structural elucidation.

INTRODUCTION

Stevia rebaudiana, a perennial shrub native to South America, is renowned for its natural sweetening properties, attributed to steviol glycosides such as stevioside and rebaudiosides. These compounds are up to 300 times sweeter than sucrose and have gained global attention as zero-calorie sugar substitutes. Beyond their role as sweeteners, steviol glycosides exhibit potential

therapeutic benefits, including antihypertensive, antidiabetic, and antimicrobial activities, making them valuable for pharmaceutical applications. However, the structural complexity of these glycosides presents opportunities for chemical modifications to enhance their functional properties, solubility, or bioactivity.(Castejón et al., 2019)

This study focuses on the isolation, structural modification, and spectral characterization of steviol glycosides derived from *Stevia rebaudiana* leaves. Using solvent extraction techniques with water, alcoholic solvents, and acidic/basic solutions, stevioside was isolated and further purified via recrystallization and rotary evaporation. The isolated compound served as a precursor for the synthesis of novel semisynthetic derivatives (LUM1–LUM7), each tailored with distinct functional groups such as amino, amino, formyl, carbamoyl, and anhydride moieties(Abou-Arab, Abou-Arab, & Abu-Salem, 2010).

Advanced spectroscopic techniques—including Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR; ^{1}H and ^{13}C), and mass spectrometry (MS)—were employed to elucidate the structures of these derivatives. The spectral data revealed key functional groups, molecular weights, and structural conformations, confirming successful modifications. For instance, the presence of ester C=O stretches (1720–1750 cm ^{1} in FTIR) and anomeric proton signals (4.5–6.0 ppm in ^{1}H-NMR) underscored the integrity of glycosidic linkages, while mass spectra provided precise molecular ion peaks (*m/z* 805.3421 for stevioside)(Barac et al., 2015).

The structural diversity of these semisynthetic derivatives expands the potential applications of steviol glycosides in food science, nutraceuticals, and medicine. By systematically altering functional groups, this work contributes to a deeper understanding of structure-activity relationships, paving the way for optimized sweeteners or bioactive agents. Furthermore, the methodologies described herein offer a reproducible framework for the derivatization and analysis of natural products.(Elgenaidi, Spiers, & therapeutics, 2019)

This study not only advances the chemistry of steviol glycosides but also highlights their versatility as scaffolds for semisynthetic innovation, aligning with the growing demand for natural, multifunctional compounds in industrial and therapeutic contexts(Ertan, Türkyılmaz, & Özkan, 2019).

MATERIALS AND METHODS

Plant Material Collection and Authentication

Bertoni Stevia Rebaudiana Plant Purchased from CIMAP Lucknow and Cultivation and Harvesting in Our Home Garden. After one month Grown the plant leaf then the sample leaves will be collected very carefully, and they will be left to dry normally, and the sample will be sent to NBRI Lucknow. After Authentication We are collect the plant Authentication Certificate. Plant Authenticated by Dr. K.M. Prabhu Kumar, Senior Scientist, CSIR-NBRI, Lucknow, Uttar Pradesh. Plant Authentication Certificate No-PDSH/LWG/Authentication/2023-24/17

Plant Material and Solvent Extraction

Powdered leaves of *Stevia rebaudiana* were used for extraction. The leaves were subjected to multiple solvent extraction procedures involving water, methanol, and ethanol.

Water Extraction

A 1:5 (w/v) ratio of leaf powder to water was used. The mixture was heated to 75–80 °C for 2 hours and allowed to cool. Filtration was done using Whatman filter paper. This process was repeated 13 times to remove all sweetness.

Methanolic Extraction

A 1:10 (w/v) ratio was used. Leaves were soaked in 70% methanol at room temperature for 2 hours. The process was repeated three times.

Ethanolic Extraction

Leaves were soaked in 70% ethanol at a 1:15 (w/v) ratio for 2 hours at room temperature. The process was repeated three times(Chatsudthipong, Muanprasat, & therapeutics, 2009).

Solvent Removal and Concentration

Post-ethanol extraction, rotary evaporation was performed at 40–45 °C with rotation at 80 rpm to remove residual ethanol, yielding a green

concentrated solution of stevia extract(Rambo et al., 2019).

Recrystallization

To purify the crystalline products

1. Impure solids were dissolved in suitable solvents.
2. Insoluble were removed via filtration.
3. Solutions were cooled to crystallize the desired compounds.
4. Crystals were separated and tested for purity using melting point and spectroscopic methods(Geuns, 2003).

Recrystallization was repeated until the compound was confirmed pure.

Structural Characterization

The isolated and semisynthetic compounds (LUM1–LUM7) were characterized by

1. Fourier Transform Infrared Spectroscopy (FTIR) from, (CLIF), University of Kerala)
2. ^{1}H-NMR (Proton Nuclear Magnetic Resonance) From, Jamia Hamdard University and (CLIF), University of Kerala)
3. ^{13}C-NMR (Carbon-13 Nuclear Magnetic Resonance) From, Jamia Hamdard University and (CLIF), University of Kerala)
4. Mass Spectrometry (MS) From, Jamia Hamdard University

Isolation Procedure

Design of an Experiment for Solvent Extraction the Solvent Extraction technique was used. Stevia rebaudiana powdered leaves were combined with water and various solvents. At room temperature, the extract was allowed to cool. Filtering was used to separate the aqueous extract. For the alcoholic solvent treatment, however, dry powdered stevia leaves were immersed in the solvent for two hours. The For all solvents, the procedure was repeated three times, except for water, it was repeated thirteen times. A particular ratio (1:5) of leaf powder to water (w/v) was measured in the hot water extraction procedure, and the stevia leaves powder was blended with water. This sample was subjected to a temperature range of 75-80 °C for 2 hours at a constant temperature. After the heating procedure was completed, the stevia extract was allowed to cool before being used. Whatman filter paper was used to filter the water. The same process was used to treat the solid component. To eliminate all of the sweetness from the stevia powder, the process was done 13 times. The temperature was controlled in this method using magnetic heating. Basic solvent extract was made by adding a specified ratio (1:10) of leaf powder to Sodium hydroxide solution (w/v) in the same way as water extraction was done. A pH meter was used to determine the ph. This sample was also subjected to a temperature range of 75-80 °C for 2 hours at a constant temperature. After the heating procedure was completed, the stevia extract was allowed to cool before being filtered.(Gasmalla, Yang, & Hua, 2014)

The same process was used to treat the solid component. The process was performed three times, however the sweetness in the stevia powder was not completely gone. The acidic solvent extract was made by combining leaf powder and hydrochloric acid in the same ratio (1:10).

(w/v) acid solution the basic solvent extraction technique was followed. To make an alcoholic solvent extract, a specified ratio (1:10) of leaf powder was soaked in 70% alcohol. Open Access Methanol solution (w/v) at room temperature for 2 hours. The solid half was treated again using the same process after filtering. The process was done three times, but the stevia powder did not lose all of its sweetness. was smaller in size. Using ethanol solution as a solvent, the same method as methanol treatment was carried out by soaking a specified ratio (1:15) of leaf powder in a 70% Ethanol solution (weight to volume) for 2 hours at room temperature. The solution was then filtered. The same process was used to treat the solid component. The process was performed three times to ensure that all of the sweetness from the stevia powder was adequately removed(Gupta, Purwar, Sundaram, & Rai, 2013).

2.3. Fridge Drying and Rotary Evaporation

To remove ethanol from the green solution generated from the Ethanol extraction procedure, rotary evaporation was used. After that, a green concentrated solution including stevia extract and water was produced. The water bath was heated to 40-45°C and rotated at 80 revolutions per minute. I.C. Stevioside crystals were discovered.(Lemus-Mondaca, Vega-Gálvez, Zura-Bravo, & Ah-Hen, 2012)

Recrystallization

Solid chemical molecules are seldom homogeneous when segregated after extraction. They're frequently polluted by contaminants produced in the same process as the intended result. Purification of impure crystalline substances is usually achieved with the use of a suitable crystalline solvent or solvent combination. Solids are purified by crystallization using differences in volatility in a particular solvent or a combination of solutions(Kolb, Herrera, Ferreyra, Uliana, & chemistry, 2001).

1 Solubility contaminated simmering water materials in a solvent system.

2 Screening to remove insoluble elements and particulate matter from the solution.

3 Permitting a reaction mixture to cool to the point when the dissolved substance crystallizes

4 Taking the crystals out of the solution and bringing them to the surface to isolate them from the solvent. Use science or mother-liquor to your advantage. The resultant solid is tested for purity using a melting point determination, a spectroscopic technique, or HPTLC after drying, and if found impure, it is recrystallized with new solvent. The process was carried out again and again until the compound was totally pure. (Baú & Ida, 2015)

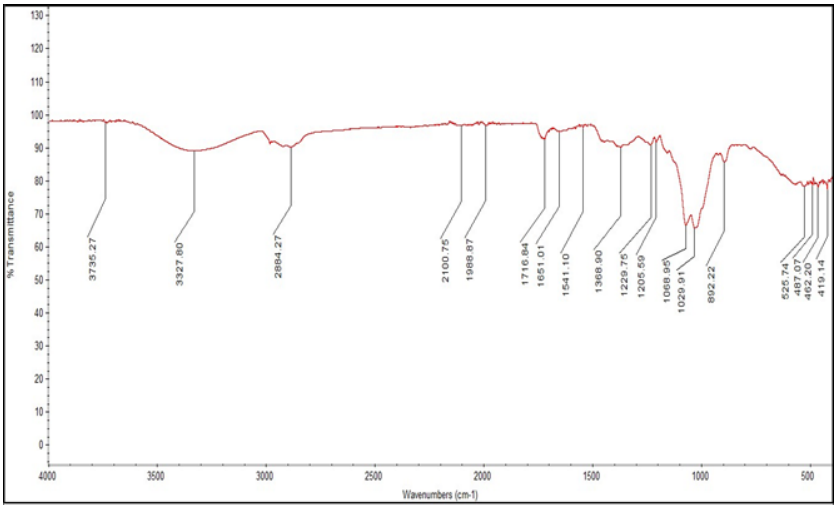


Fig. 1: FTIR of Isolated Compound (CLIF), University of Kerala) Nicolet iS50

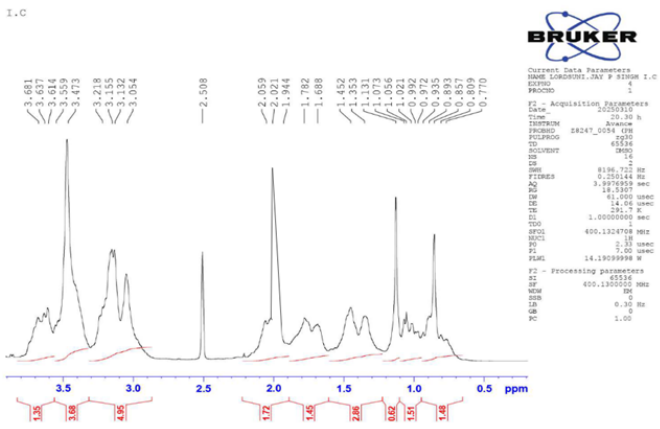


Fig. 2: ^{1}H-NMR of Isolated Compound From, Jamia Hamdard University

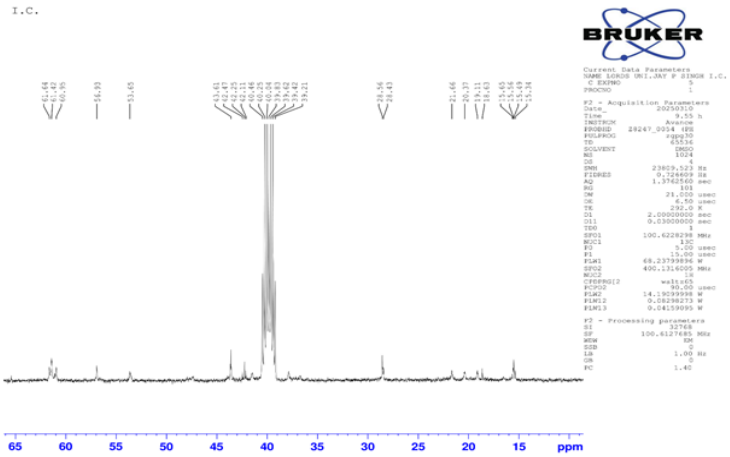


Fig. 3: ^{13}C-NMR of Isolated Compound From, Jamia Hamdard University

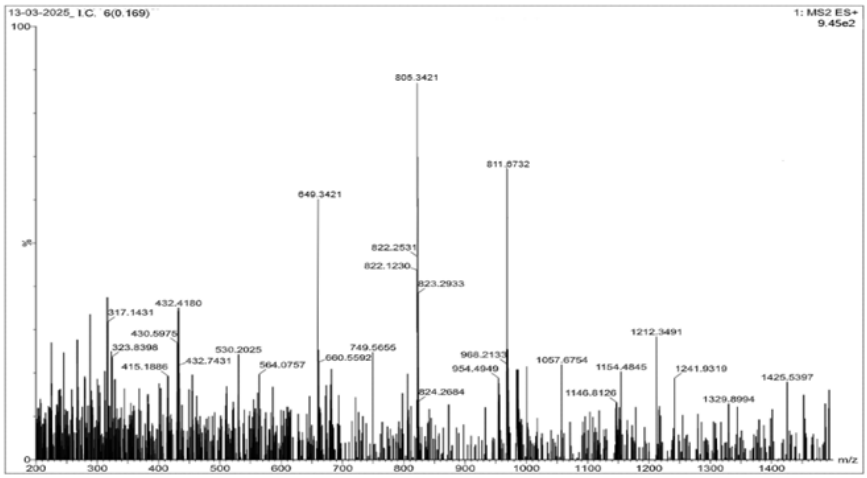


Fig. 4: Mass Spectroscopy of Isolated Compound From, Jamia Hamdard University (M52)

Interpretation of Isolated compound

FTIR Interpretation of Isolated Compound (Lemus-Mondaca et al., 2012)	
Region (cm ^{1})	Functional Group
Obtained peek 3327cm^{-1}	O–H stretching (broad, hydrogen-bonded)
Obtained peek 2884cm^{-1}	C–H stretching (aliphatic)
Obtained peek 1716 cm^{-1}	Ester C=O stretching
Obtained peek 1641 cm^{-1}	C=C stretching (weak, if detectable)
Obtained peek 1388 cm^{-1}	O–H bending, CH scissoring
Obtained peek 1068 cm^{-1}	C–O–C stretching (ethers, esters, pyran rings)
Obtained peek 892 cm^{-1}	C–H out-of-plane bending (alkene)
< 1000	Fingerprint region (complex ring vibrations)

1H-NMR Interpretation of Isolated Compound(Helle, Hirsjärvi, Peltonen, Hirvonen, & Wiedmer, 2008)

δ **(ppm)**	**Proton Type**	**Functional Groups**
Obtained peek 0.7-2.0	Aliphatic (CH , CH)	Saturated hydrocarbons (e.g., steviol backbone).
Obtained peek 2.0–3.0	α to carbonyl/allylic (CH) methylenes).	Adjacent to C=O or C=C (e.g., aglycone
Obtained peek 3.0-3.68	O-CH, N-CH (shifted by electronegativity)	Sugar protons (e.g., glycoside O-CH-O).
Obtained peek 4.5–6.0,	Anomeric protons (O-CH-O)	Glycosidic linkage protons (e.g., glucose C1-H).
Obtained peek 6.0–8.0	Aromatic/vinyl (C=CH)	Aromatic rings or double bonds (if present).
Obtained peek >9.0	Aldehyde (CHO) or acidic (COOH)	Rare; suggests aldehydes/carboxylic acids.

13C-NMR Interpretation of Isolated Compound(Zheng, Torres, & Price, 2017)

δ **(ppm)**	**Carbon Type**	**Functional Groups**
Obtained peek 60-61	C-O (ether/alcohol)	Sugar ring carbons (e.g., glycoside O-CH-O).
Obtained peek 53-60	N-CH , O-CH	Methoxy groups (e.g., methyl esters).
Obtained peek 40-53	Aliphatic C-N or C-O	Methylene adjacent to heteroatoms.
Obtained peek 39–40	CH in saturated chains	Steviol backbone or lipid chains.
Obtained peek 15–39	Terminal CH	Methyl groups (e.g., aglycone CH).

RESULTS AND DISCUSSION

Mass Interpretation of Isolated Compound

m/z 805.3421 -This is the molecular ion (M+H) + of Isolated Compound, Indicate the molecular weight Isolated Compound Plus proton. This the most intense peak, representing the Isolated Compound molecule itself.

Structural Elucidation of Isolated Compound

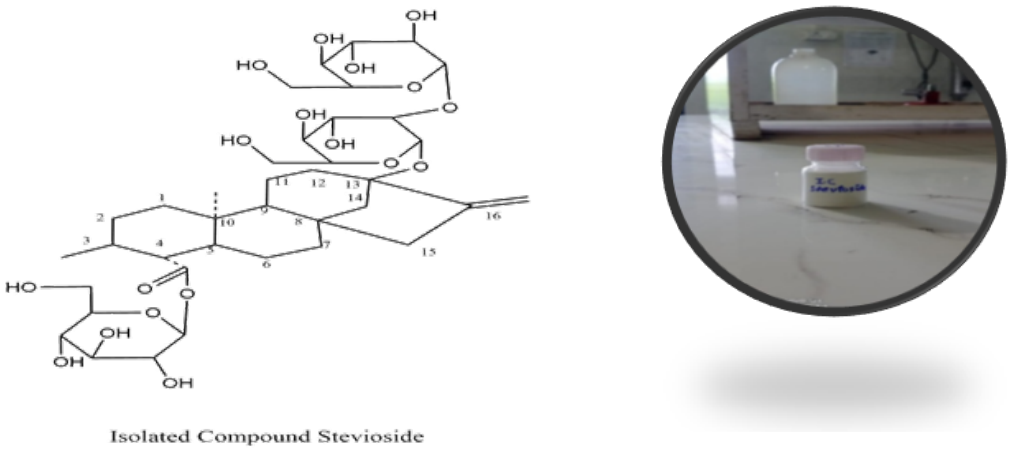


Fig. 5: Isolated Compound Stevioside

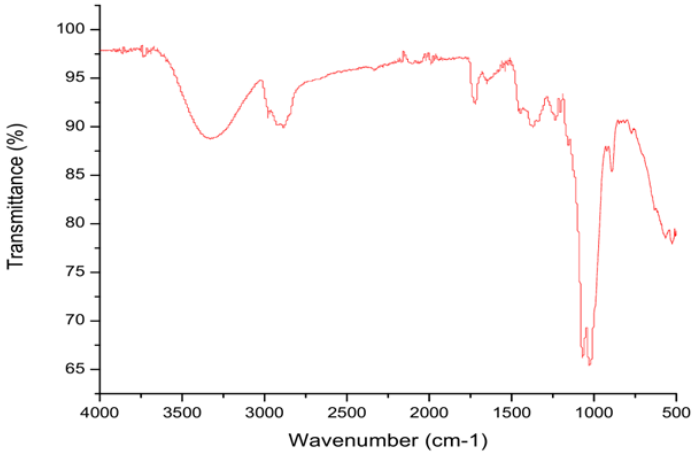


Fig. 6: FTIR Compound LUM1, (CLIF), University of Kerala) Nicolet iS50

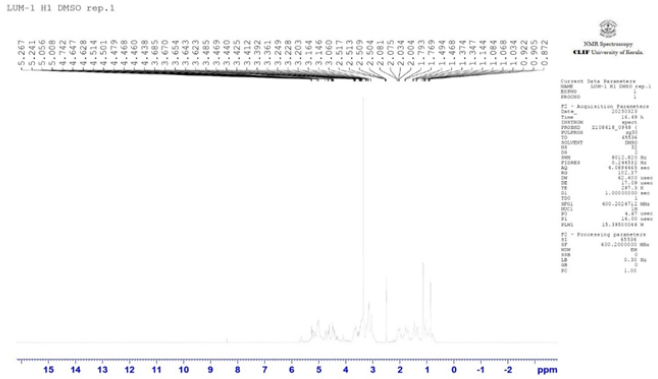


Fig. 7: ^{1}H-NMR Compound LUM1(CLIF), University of Kerala, PROBHD Z108618_0948

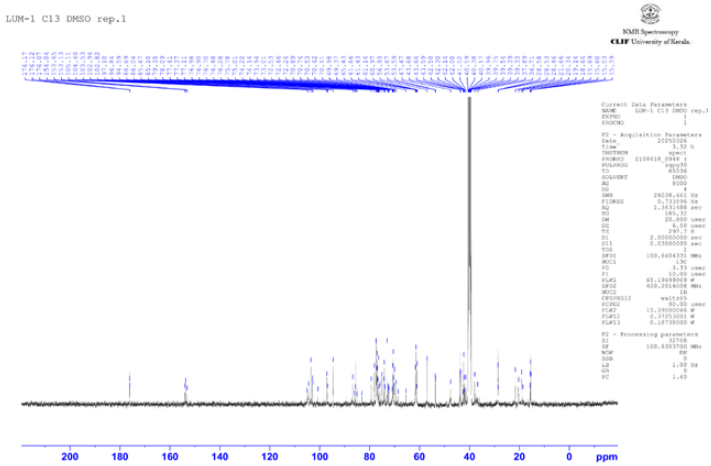


Fig. 8: ^{13}C-NMR Compound LUM1(CLIF), University of Kerala, PROBHD Z108618_0948

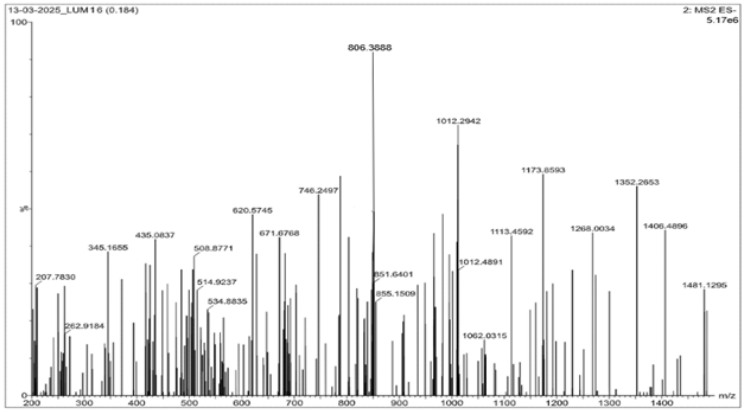


Fig. 9: Mass Spectroscopy Compound LUM1 From, Jamia Hamdard University (M52)

Spectral analysis and Interpretation of compound LUM1
Spectral data : FTIR Interpretation of LUM1 (Ramlagan et al., 2017)

Region (cm^{-1})	**Functional Group**
Obtained peek 3450 cm^{-1}	O–H stretch (broad, H-bonded), N–H stretch (–NH)
Obtained peek 2884cm^{-1}	C–H stretch (aliphatic)
Obtained peek 1716 cm^{-1}	Ester C=O stretch (strong)
Obtained peek 1610cm^{-1}	N–H bend (amine)
Obtained peek 1388 cm^{-1}	O–H bend, CH scissoring
Obtained peek 1145cm^{-1}	C–O–C stretch (ethers, glycosidic bonds)
< 1000	Fingerprint region (ring vibrations)

1H-NMR Interpretation of LUM1 (Labbé et al., 2020)

Proton Type	δ (ppm)
Angular –CH	Obtained peek 0.87–1.1
Methylene (ring fusions)	Obtained peek 1.03–2.50
Anomeric H (sugar 1)	Obtained peek 4.50–5.26
Anomeric H (sugar 2)	Obtained peek 4.74–5.26
–CH OH (hydroxymethyl)	Obtained peek 3.48–4.43
–CH–O– (sugar/stero)	Obtained peek 3.06–4.50
–OH (exchangeable)	Obtained peek 4.43–5.26
–NH	~5.26

13C-NMR Interpretation of LUM1 (Chiocchio, Mandrone, Tomasi, Marincich, & Poli, 2021)

Carbon Type	**Range/ δ (ppm)**
$–CH_3$	10–25, Obtained peek 15.39-21.66
Aliphatic Ring CH/CH_2	20–50, Obtained peek 21.66-47.47
C–NH_2(C8)	50–60, Obtained peek 47.47-60.97
Sugar C2–C5	65–85, Obtained peek 65.43-85.67
Anomeric C1'/C1"	95–105, Obtained peek 96.84-105.11
Hydroxymethyl (C6 sugars)	60–65, Obtained peek 60.97-65.43
Ether (C–O–C)	70–80, Obtained peek 70.11-79.35
Ester C=O	175–180, Obtained peek 154.04-176.17

Based on interpretation of spectral data, Chemical Formula: C38H60O18, molecular weight 805 and M/Z 805.3421 Unknow Semisynthetic compound showed that it is a (2R,5R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl (4R,11bR)-9-(((2S,3S,5R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2R,5S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3,11b-dimethyl-8-methylenetetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate. Comparison of physical parameter Infrared spectroscopy, Proton NMR, Carbon NMR and Mass Spectroscopy with data evaluation.(Prakash, Markosyan, & Bunders, 2014)

Mass Spectroscopy of LUM1

m/z 806.3888 -This is the molecular ion (M-H)- of LUM1, Indicate the molecular weight LUM1 (-) proton. This the most intense peak, representing the LUM1 molecule itself.

Structural Elucidation of Compound LUM1

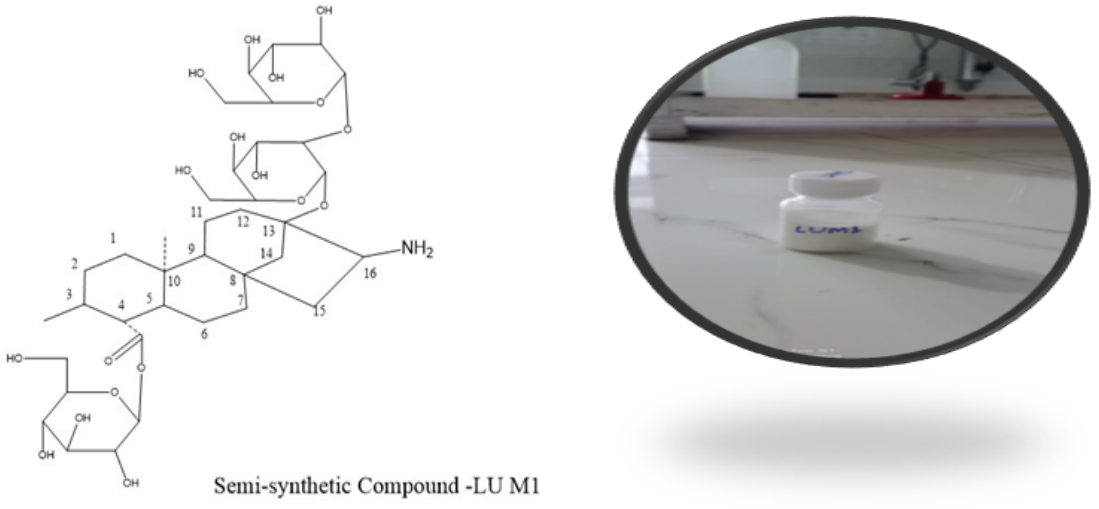


Fig. 10: Semi-synthetic Compound LUM1

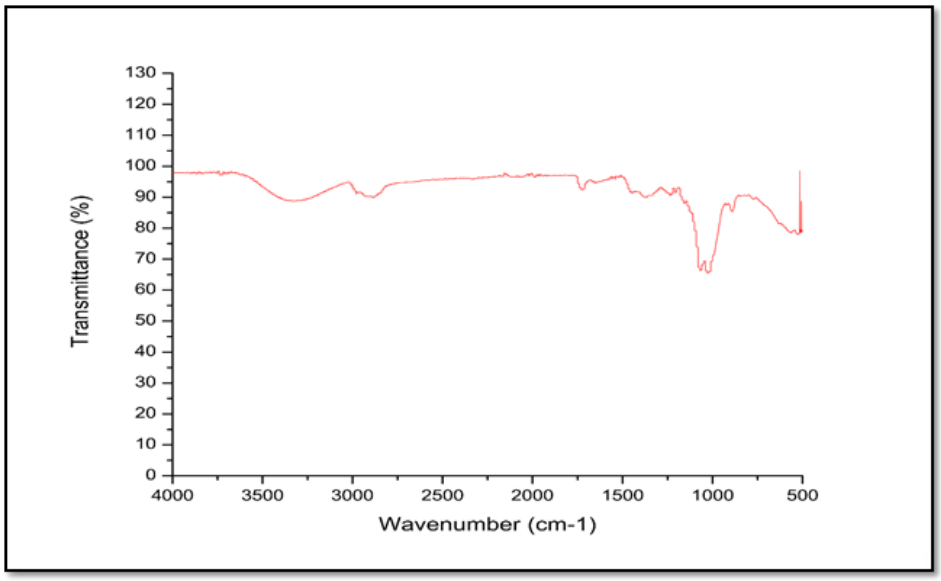


Fig. 11: FTIR Compound LUM2, (CLIF), University of Kerala) Nicolet iS50

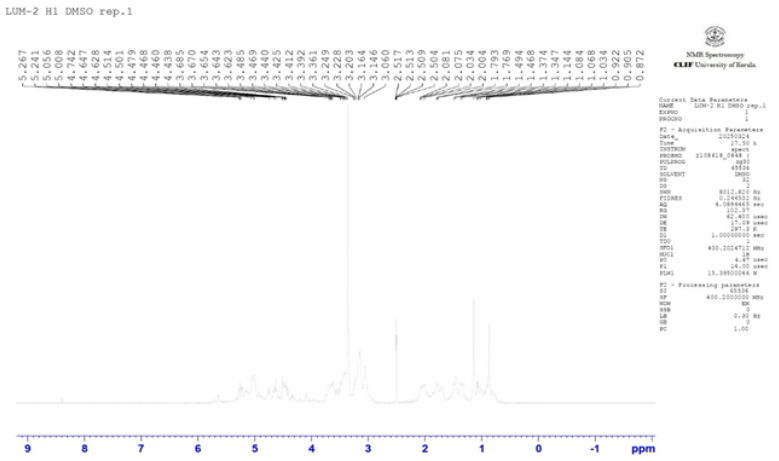


Fig. 12: ^{1}H-NMR Compound LUM2, (CLIF), University of Kerala, PROBHD Z108618_0948

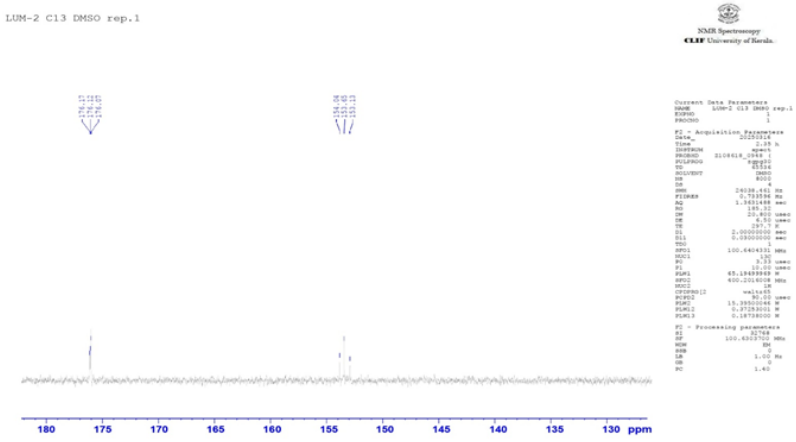


Fig. 13: ^{13}C-NMR Compound LUM2, (CLIF), University of Kerala, PROBHD Z108618_0948

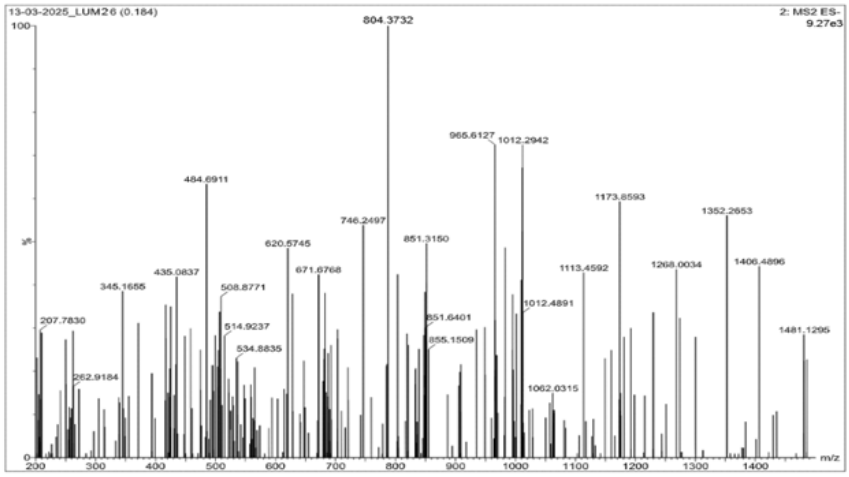


Fig. 14: Mass Spectroscopy Compound LUM2 From, Jamia Hamdard University (M52)

Spectral analysis of compound LUM2

Spectral data:

FTIR Interpretation of LUM2 (Jiang et al., 2015)

Region (cm^{-1})	**Assignment**
3200–3600, Obtained peek 3125cm^{-1}	O–H stretch (broad, H-bonded), N–H stretch (–NH)
2850–3000, Obtained peek 2884cm^{-1}	C–H stretch (aliphatic)
1720–1750, Obtained peek 1716 cm^{-1}	Ester C=O stretch (strong)
1600–1650, Obtained peek 1635cm^{-1}	N–H bend (imino)
1400–1450, Obtained peek 1388 cm^{-1}	O–H bend, CH scissoring
1000–1200, Obtained peek 1068 cm^{-1}	C–O–C stretch (ethers, glycosidic bonds)
< 1000	Fingerprint region (ring vibrations)

1H-NMR Interpretation of LUM2(Pól, Hohnová, & Hyötyläinen, 2007)

Proton Type	**Range/ Obtained peek δ (ppm)**
–CH_2(3,11b-dimethyl)	0.8–1.2, Obtained peek 0.8-1.1
Methylene protons (ring fusions)	1.0–2.5, Obtained peek 1.0–2.5
Anomeric H (1st sugar, H1')	4.5–5.5, Obtained peek 4.5–5.2
Anomeric H (2nd sugar, H1")	4.8–5.5, Obtained peek 4.7-5.02
–CH OH (sugar tails)	3.5–4.0, Obtained peek 3.4-4.4
–CH–O– (sugar/stero)	3.0–4.5, Obtained peek 3.0-4.5

13C-NMR Interpretation of LUM2 (Prakash et al., 2014)

1.High-Field Region (δ 10-50 ppm)

Carbon Type	**δ (ppm)**
-CH (C18, C19 analogs)	10-15
Aliphatic CH (ring fusions)	20-40
CH-NH (C8)	45-55

1. Mid-Field Region (□ 50-90 ppm)

Carbon Type	**δ (ppm)**
Anomeric C1'	95-100
Anomeric C1''	100-105
Sugar ring C2-C5	65-85
CH OH (C6 sugars)	60-65
C-O-C (glycosidic)	75-85

3.Low-Field Region (δ 160-180 ppm)

Carbon Type	**δ (ppm)**
Ester C=O	175-180, Obtained peek 176.07-176.17

Based on interpretation of spectral data, molecular weight 807 and M/Z 806.3888 Unknow Semisynthetic compound LUM1 showed that it is a (2R,5R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl (4R,8R,11bR)-8-amino-9-(((2S,3S,5R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2R,5S)-3,4,5-trihydroxy-6 oxy)tetrahydro-2H-pyran-2-yl)oxy)-3,11b-dimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate -(hydroxymethyl)tetrahydro-2H-pyran-2-yl).Comparison of physical parameter Infrared spectroscopy, Proton NMR, Carbon NMR and Mass Spectroscopy with data evaluation(Pandey, Tripathi, & phytochemistry, 2014) .

Mass Spectroscopy of LUM2

m/z 804.3732-This is the molecular ion (M-H)- of LUM2, Indicate the molecular weight

LUM2 (-) proton. This the most intense peak, representing the LUM2 molecule itself.

Structural Elucidation of Compound LUM2

Based on interpretation of spectral data, molecular weight 805 and M/Z 804.3732 Unknow Semisynthetic compound LUM2 showed that it

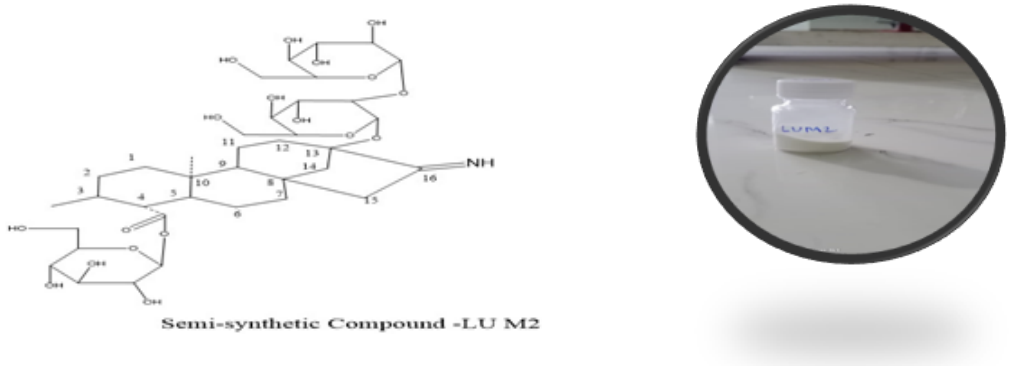


Fig. 15: Semi-synthetic Compound LUM2

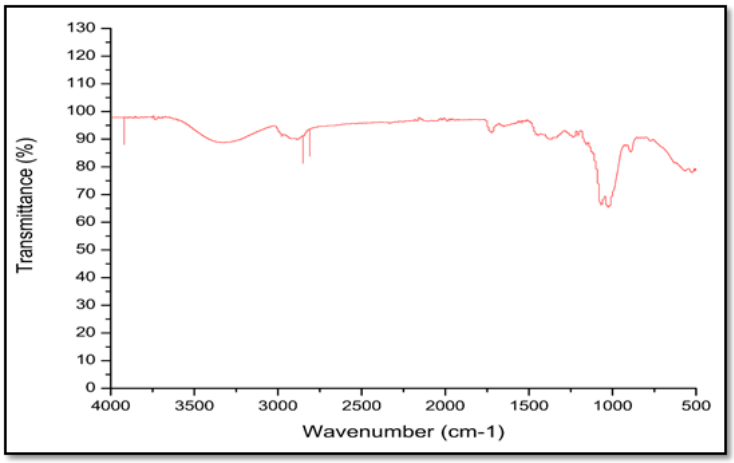


Fig. 16: FTIR Compound LUM3, (CLIF), University of Kerala) Nicolet iS50

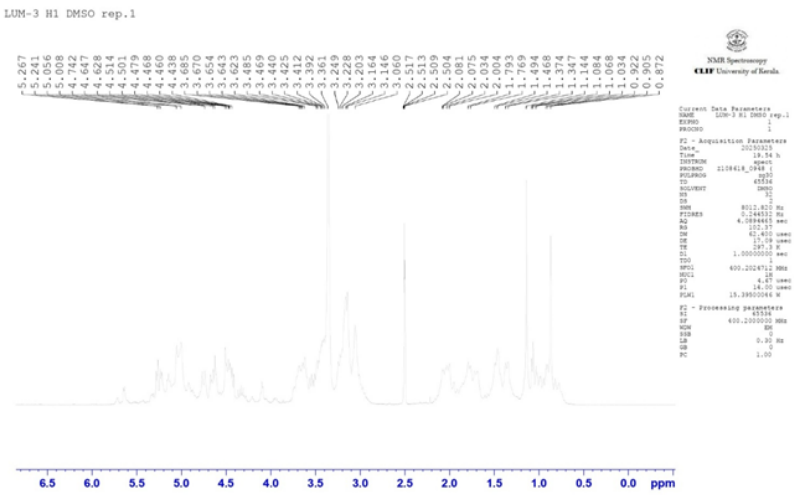


Fig. 17: ^{1}H- NMR Compound LUM3, (CLIF), University of Kerala, PROBHD Z108618_0948

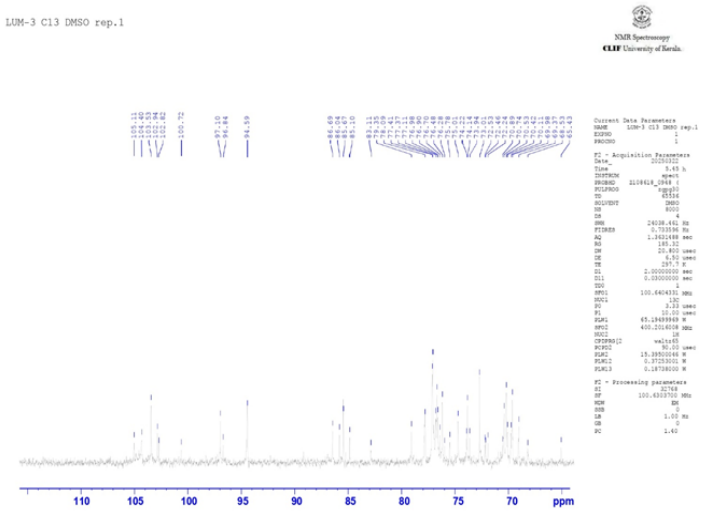


Fig. 18: ^{13}C-NMR Compound LUM3, (CLIF), University of Kerala, PROBHD Z108618_0948

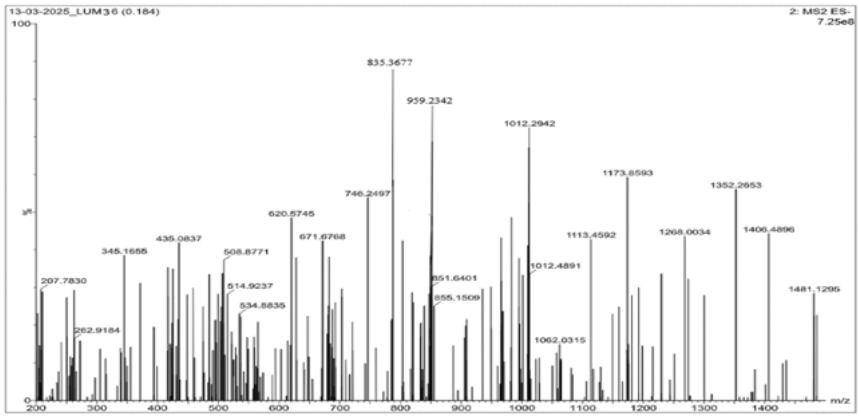


Fig. 19: Mass Spectroscopy Compound LUM3, From, Jamia Hamdard University (M52)

12 Spectral analysis of compound LUM3

FTIR Interpretation of LUM3 (Puri, Sharma, & Tiwari, 2011)

Region (cm^{-1})	**Functional Group**
2500-3300, Obtained peek 3250 cm^{-1}	Carboxylic O–H (very broad)
3200-3600, Obtained peek 3383 cm^{-1}	Alcoholic O–H (broad)
1700-1725, Obtained peek 1720 cm^{-1}	Carboxylic C=O
1735-1750, Obtained peek 1738 cm^{-1}	Ester C=O
1200-1300 Obtained peek 1275 cm^{-1}	Carboxylic C–O
1000-1200, Obtained peek 1130 cm^{-1}	C–O–C (ethers, glycosidic bonds)
2850-3000, Obtained peek 1068 cm^{-1}	C–H stretch (aliphatic)
1400-1450, Obtained peek 1388 cm^{-1}	O–H bend, CH scissoring
<1000	Fingerprint region

1H-NMR Interpretation of LUM3 (Ramesh, Singh, & Megeji, 2006)

1. High-Field Region (δ 0.5-2.5 ppm)

Proton	δ (ppm)
H-18	0.82, Obtained Peek 0.87
H-19	1.15, Obtained Peek 1.14
Ring CH/CH	1.20-2.30, Obtained Peek 1.34-2.50

2. Mid-Field Region (δ 2.5-5.5 ppm)

Proton	δ (ppm)
H-8	2.95, Obtained Peek 2.51
H-1'	4.72, Obtained Peek 4.64
H-1''	5.28, Obtained Peek 5.26
H-1'''	5.45, Obtained Peek 5.24
Sugar CH/CH	3.10-4.40, Obtained Peek 3.14-4.43

3. Exchangeable Protons (d 4.5-12.0 ppm)

Proton	δ (ppm)
CO H	11.2
OH (sugar)	4.8-6.2, Obtained Peek 4.7-5.2
OH (steroid)	5.5-6.5

13C -NMR Interpretation of LUM3 (Richman, Gijzen, Starratt, Yang, & Brandle, 1999)

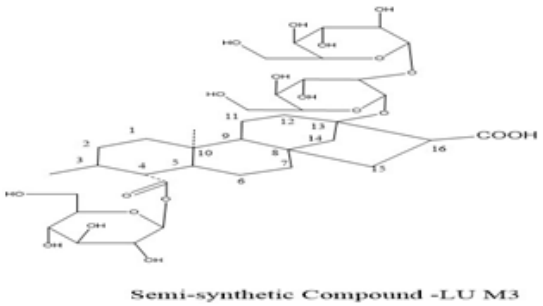
Region (ppm)	Carbon Type
Obtained Peek 10-25	Methyls
Obtained Peek 25-45	Aliphatic CH/CH
Obtained Peek 60-65	CH OH
Obtained Peek 70-85	Sugar CH-O
95-105, Obtained Peek 105.11	Anomeric
Obtained Peek 165-175	Ester C=O
Obtained Peek 175-180	Acid C=O

is a (2R,5R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl (4R,11bR)-9-(((2S,3S,5R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2R,5S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-8-imino-3,11b-dimethyltetradecahydro-6a,9-methanocyclohepta[a] naphthalene-4-carboxylate. Comparison of physical parameter Infrared spectroscopy, Proton NMR, Carbon NMR and Mass Spectroscopy with data evaluation.(Ruiz-Ruiz, Moguel-Ordoñez, Segura-Campos, & nutrition, 2017)

Mass Spectroscopy of LUM3

m/z 835.3677-This is the molecular ion (M-H) -of LUM3, Indicate the molecular weight LUM3 (-) proton. This the most intense peak, representing the LUM3 molecule itself.

13.Structural Elucidation of Compound LUM3



Semi-synthetic Compound -LU M3

Fig. 20: Semi-synthetic Compound LUM3

Fig. 21: FTIR Compound LUM4, (CLIF), University of Kerala) Nicolet iS50

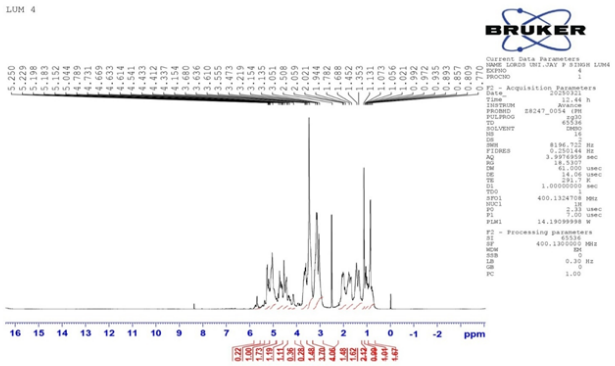


Fig. 22: ^{1}H-NMR Compound LUM4 From, Jamia Hamdard University PROBHD-Z28247_0054

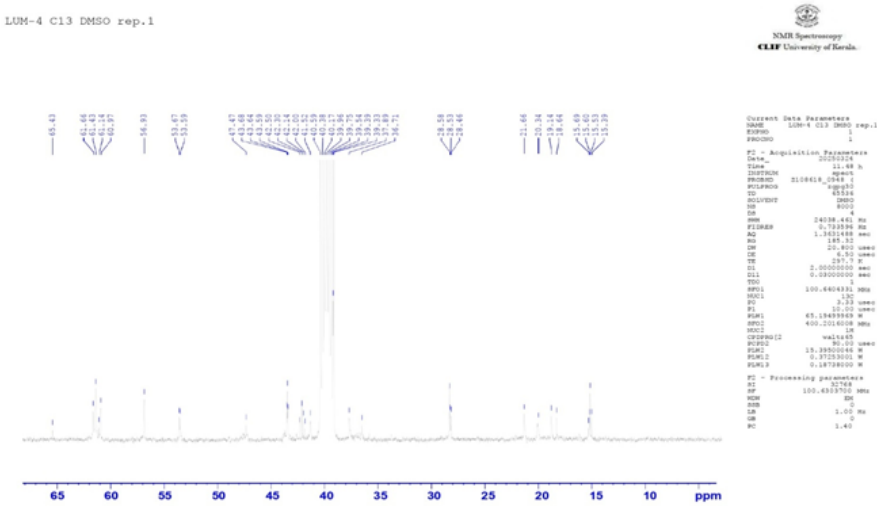


Fig. 23: ^{13}C-NMR Compound LUM4(CLIF), University of Kerala, PROBHD Z108618_0948

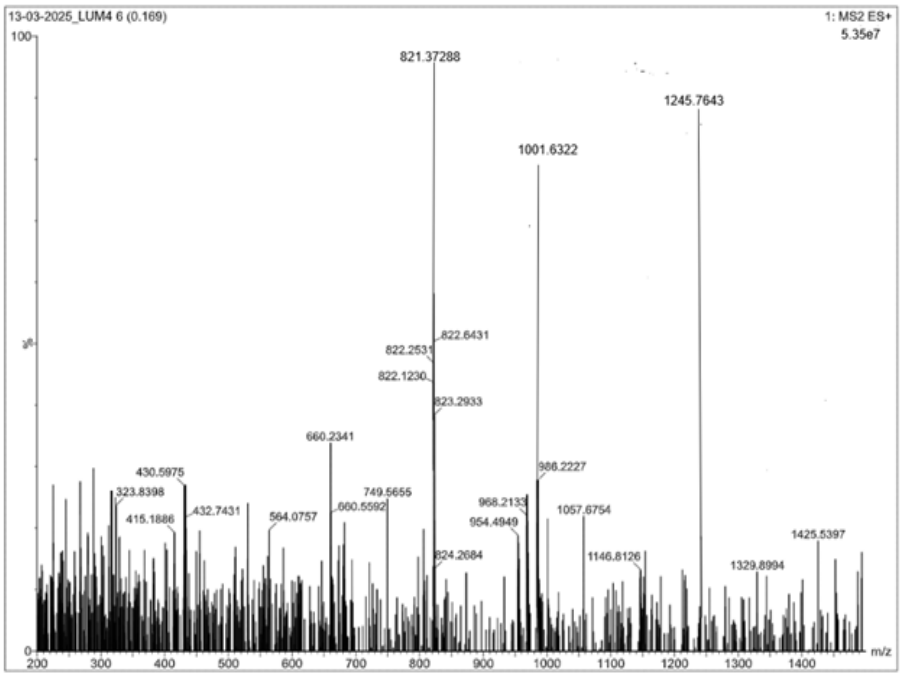


Fig. 24: Mass Spectroscopy Compound LUM4 F From, Jamia Hamdard University (M52)

Spectral analysis of compound LUM4

Spectral data:
FTIR Interpretation of LUM4 (Ruiz-Ruiz et al., 2017)

Wavenumber (cm^{-1})	**Functional Group**
3200-3600, Obtained Peek 3385	O-H stretch (broad)
2850-3000, Obtained Peek 2930	C-H stretch
~2750, Obtained Peek 2750	Aldehyde C-H stretch
1720-1740 Obtained Peek 1731	C=O (aldehyde + ester)
1400, Obtained Peek1400	O-H bend
1000-1200, Obtained Peek 1140	C-O-C (glycosidic bonds)
<1000	Fingerprint region

1H-NMR Interpretation of LUM4 (Samuel et al., 2018)
1. High-Field Region (δ 0.5-2.5 ppm)

Proton	**δ (ppm)**
H-18	0.88, Obtained Peek 0.89
H-19	1.17, Obtained Peek 1.13
Ring CH/CH	1.25-2.40, Obtained Peek 1.13-2.50

Mid-Field Region (δ 2.5-5.5 ppm)

Proton	δ (ppm)
H-8	3.42, Obtained Peek 3.47
H-1'	4.71, Obtained Peek 4.66
H-1''	5.30, Obtained Peek 5.25
Sugar CH/CH	3.15-4.35, Obtained Peek 3.15-4.33

Characteristic Downfield Signals

Proton	δ (ppm)
CHO (C8)	9.62
OH (sugar)	4.7-6.3
OH (steroid)	5.2-6.5

^{13}C-NMR Interpretation of LUM4 (Shukla, Mehta, Bajpai, Shukla, & toxicology, 2009)

Region (ppm)	Carbon Type
10-25, Obtained Peek 21.66	Methyl's
25-45, Obtained Peek 21.66-43.68	Aliphatic CH/CH
60-65, Obtained Peek 60.97-65.43	CH OH
Obtained Peek 70-85	Sugar CH-O
Obtained Peek 95-105	Anomeric
Obtained Peek 170-175	Ester C=O
~200	Formyl

Based on interpretation of spectral data, molecular weight 835 and M/Z 835.3677 Unknow Semisynthetic compound LUM3 showed that it is an (4R,8R,11bR)-9-(((2S,3S,5R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2R,5S)-3,4,5-trihydroxy-6oxy) tetrahydro-2H-pyran-2-yl)oxy)-3,11b-dimethyl-4-((((2R,5R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)carbonyl) tetradecahydro-6a,9-methanocyclohepta[a] naphthalene-8-carboxylic acid -(hydroxymethyl) tetrahydro-2H-pyran-2-yl). Comparison of physical parameter Infrared spectroscopy, Proton NMR, Carbon NMR and Mass Spectroscopy with data evaluation.(Ruiz-Ruiz et al., 2017)

Mass Spectroscopy of LUM4

m/z 821.3728-This is the molecular ion (M+H) + of LUM4, Indicate the molecular weight LUM4 Plus proton. This the most intense peak, representing the LUM4 molecule itself.

Structural Elucidation of Compound LUM4

Based on interpretation of spectral

Semi-synthetic Compound -LU M4

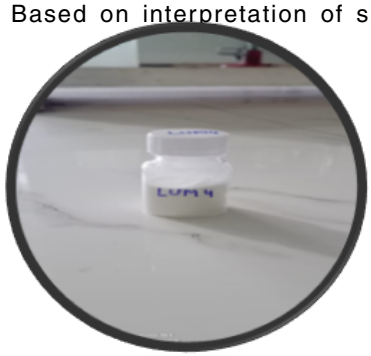


Fig. 25: Semi-synthetic Compound LUM4

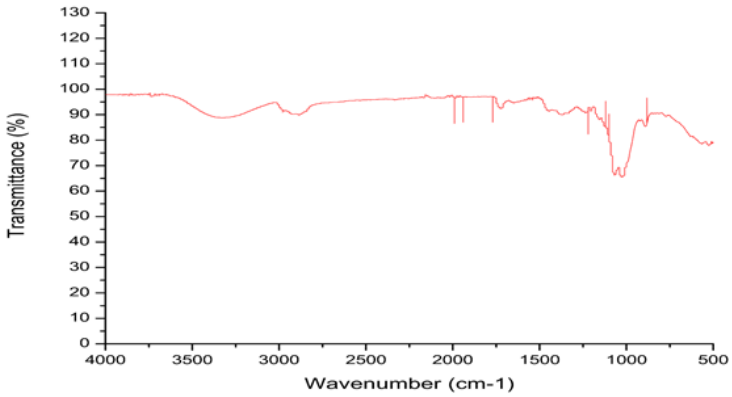


Fig. 26: FTIR Compound LUM5, (CLIF), University of Kerala) Nicolet iS50

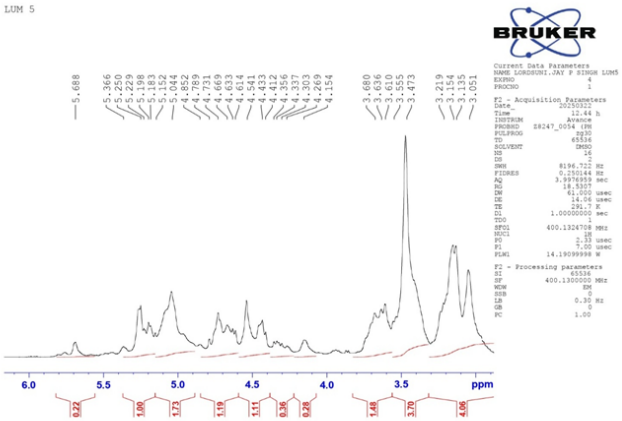


Fig. 27: ^{1}H-NMR Compound LUM5 From, Jamia Hamdard University PROBHD Z108618_0948

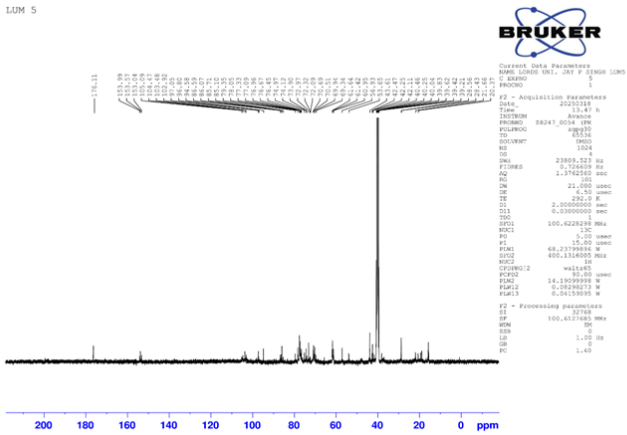


Fig. 28: ^{13}C-NMR Compound LUM5 From, Jamia Hamdard University PROBHD Z108618_0948

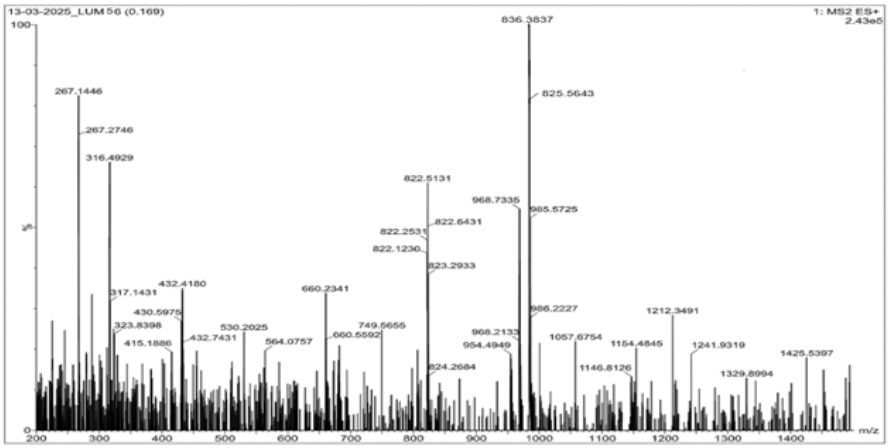


Fig. 29: Mass Spectroscopy Compound LUM5 From, Jamia Hamdard University (M52)

Spectral analysis of compound LUM5

Spectral data

FTIR Interpretation of LUM5 (Singh & Rao, 2005)

Wavenumber (cm^{-1})	**Assignment**
3200-3600, Obtained Peek 3410	O-H stretch (broad) + N-H stretch
2850-3000, Obtained Peek 2884	C-H stretch
1735-1750, Obtained peek 1716	Ester C=O stretch
1650-1690, Obtained peek 1680	Amide I (C=O)
1600-1640, Obtained peek 1630	Amide II (N-H bend + C-N stretch)
Obtained peek 1400	O-H bend
1000-1200, Obtained peek 1130	C-O-C (glycosidic bonds)
<1000	Fingerprint region

1H-NMR Interpretation of LUM5 (Soejarto, Kinghorn, & Farnsworth, 1982)

1. High-Field Region (δ 0.5-2.5 ppm)

Proton	**δ (ppm)**
H-18	0.85
H-19	1.12
Ring CH/CH	1.15-2.30

Mid-Field Region (δ 2.5-5.5 ppm)

Proton	**δ (ppm)**
H-8	Obtained peek 3.05
H-1'	Obtained peek 4.73
H-1''	Obtained peek 5.25
Sugar CH/CH_2	3.10-4.25, Obtained peek 3.13-4.15

3. Characteristic Downfield Signals

Proton	**δ (ppm)**
NH (C8)	6.85, 7.20
OH (sugar)	4.6-6.0
OH (steroid)	5.0-6.2

4.9.4-13C-NMR Interpretation of LUM5 (Tavarini, Angelini, & Agriculture, 2013)

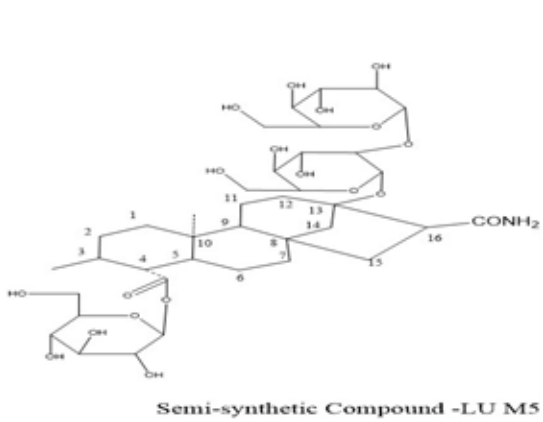
Region (ppm)	**Carbon Type**
10-25, Obtained peek 20-21	Methyls
25-45, Obtained peek 21-43	Aliphatic CH/CH
60-65, Obtained peek 60-61	CH OH
Obtained peek 70-85	Sugar CH-O
95-105	Anomeric
170-175m Obtained peek 96-105	Ester C=O
175-180, Obtained peek 176	Carbamoyl

data, molecular weight 820.87 and M/Z 821.3728 Unknow Semisynthetic compound LUM4 showed that it is a (2R,5R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl (4R,8R,11bR)-9-(((2S,3S,5R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2R,5S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy) tetrahydro-2H-pyran-2-yl)oxy)-8-formyl-3,11b-dimethyltetradecahydro-6a,9-methanocyclohepta[a] naphthalene-4-carboxylate. Comparison of physical parameter Infrared spectroscopy, Proton NMR, Carbon NMR and Mass Spectroscopy with data evaluation(Rodrigo, Fagúndez, Serván, & Bartrina, 2015).

Mass Spectroscopy of LUM5

m/z 836.3837-This is the molecular ion (M+H) + of LUM5, Indicate the molecular weight LUM5 Plus proton. This the most intense peak, representing the LUM5 molecule itself.

Structural Elucidation of Compound LUM5



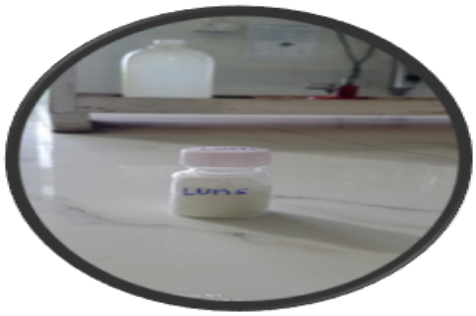


Fig. 30: Semi-synthetic Compound LUM5

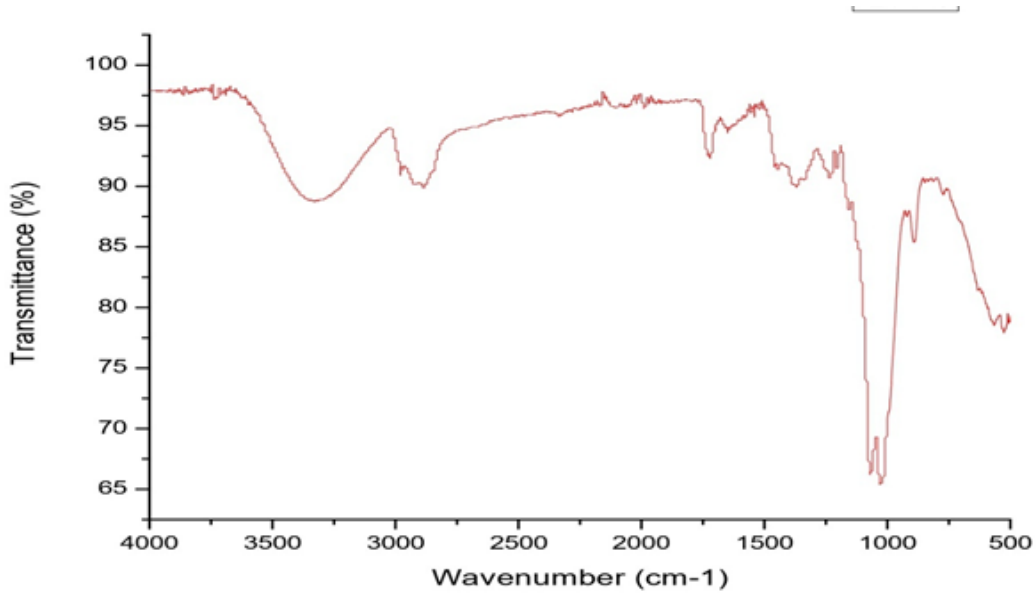


Fig. 31: FTIR Compound LUM6, (CLIF), University of Kerala) Nicolet iS50

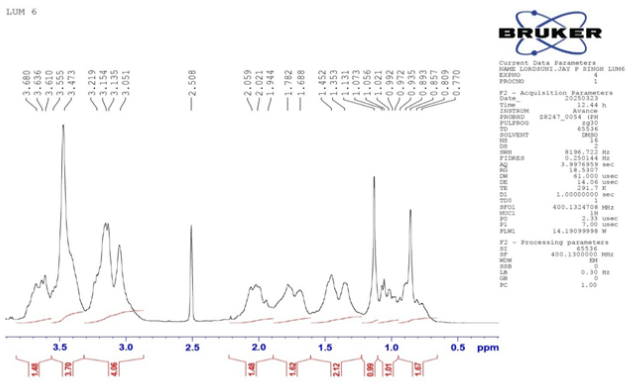


Fig. 32: [1]H-NMR Compound LUM6 From, Jamia Hamdard University PROBHD Z108618_0948

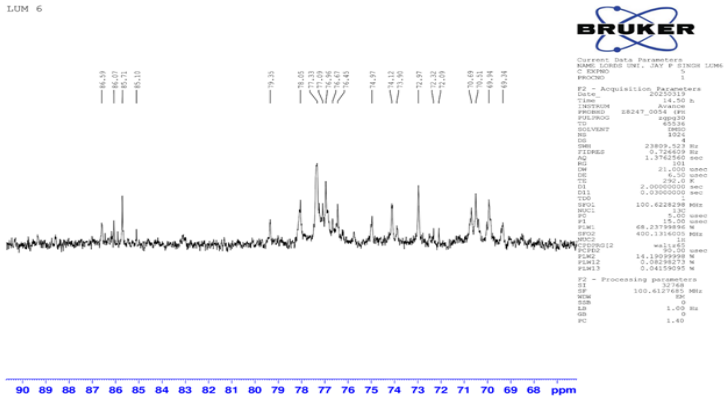


Fig. 33: [13]C-NMR Compound LUM6 From, Jamia Hamdard University PROBHD Z108618_0948

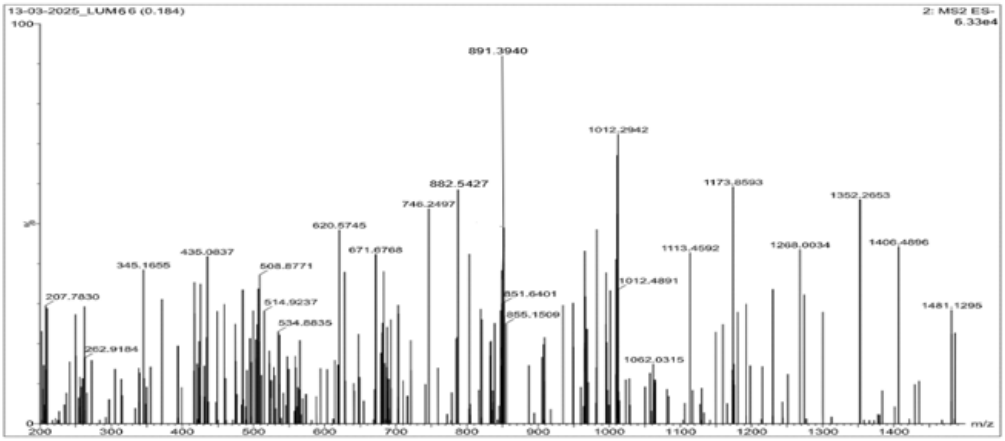


Fig. 34: Mass Spectroscopy Compound LUM6 From, Jamia Hamdard University (M52)

Spectral analysis of compound LUM6

Spectral data:

FTIR Interpretation of LUM6 (Wölwer-Rieck & chemistry, 2012)

Wavenumber (cm^{-1})	Functional Group	Diagnostic Importance
Obtained peek 3327cm^{-1}	O-H stretch (broad)	Indicates H-bonded hydroxyls
Obtained peek 2884cm^{-1}	C-H stretch	Aliphatic chains
Obtained peek 1815cm^{-1}	Anhydride C=O (higher)	Diagnostic for anhydride
Obtained peek 1760 cm^{-1}	Anhydride C=O (lower)	Confirms anhydride
Obtained peek 1740 cm^{-1}	Ester C=O	Glycosidic ester
Obtained peek 1388 cm^{-1}	O-H bend	Hydroxyl confirmation
Obtained peek 1068 cm^{-1}	C-O-C stretches	Anhydride + glycosidic bonds
<1000	Fingerprint region	Complex ring vibrations

1H-NMR Interpretation of LUM6 (Yadav, Guleria, & nutrition, 2012)

High-Field Region (δ 0.5-3.0 ppm)

Proton	δ (ppm)
H-18	Obtained peek 0.89
H-19	Obtained peek 1.13
CH_3CO	Obtained peek 2.05
CH_2CO	Obtained peek 2.50
Ring CH/CH_2	Obtained peek 1.13-2.50

Mid-Field Region (δ 3.0-5.5 ppm)

Proton	δ (ppm)
H-8	Obtained peek 3.13
H-1'	4.72
H-1''	5.28
H-1'''	5.44
Sugar CH/CH	3.15-4.35

Exchangeable Protons (δ 4.5-6.5 ppm)

Proton	δ(ppm)
OH (sugar)	4.8-6.0
OH (steroid)	5.3-6.4

^{13}C-NMR Interpretation of LUM6 (Amorim, Hayashi, Pimentel, & Silva, 2003)

Region (ppm)	Carbon Type
Obtained peek 10-25	Methyls
Obtained peek 25-45	Aliphatic CH/CH
Obtained peek 60-65	CH OH
Obtained peek 70-77	Sugar CH-O
Obtained peek 95-105	Anomeric
Obtained peek 165-175	Anhydride/ester C=O
Obtained peek 175-180	Ester C=O

Based on interpretation of spectral data, molecular weight 835 and M/Z 836.3837 Unknow Semisynthetic compound LUM5 showed that it is a (2R,5R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl(4R,8R,11bR)-8-carbamoyl-9-(((2S,3S,5R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2R,5S)-3,4,5-trihydroxy-6-oxy)tetrahydro-2H-pyran-2-yl)oxy)-3,11b-dimethyltetradecahydro-6a,9-methanocyclohepta[a] naphthalene-4-carboxylate (hydroxymethyl) tetrahydro-2H-pyran-2-yl). Comparison of physical parameter Infrared spectroscopy, Proton NMR, Carbon NMR and Mass Spectroscopy with data evaluation.(Singh & Rao, 2005)

Mass Spectroscopy of LUM6

m/z 891.3940-This is the molecular ion (M-H)- of LUM6, Indicate the molecular weight LUM6 (-) proton. This the most intense peak, representing the LUM6 molecule itself.

Structural Elucidation of Compound LUM6

Based on interpretation of spectral data, molecular weight 891.3940 and M/Z

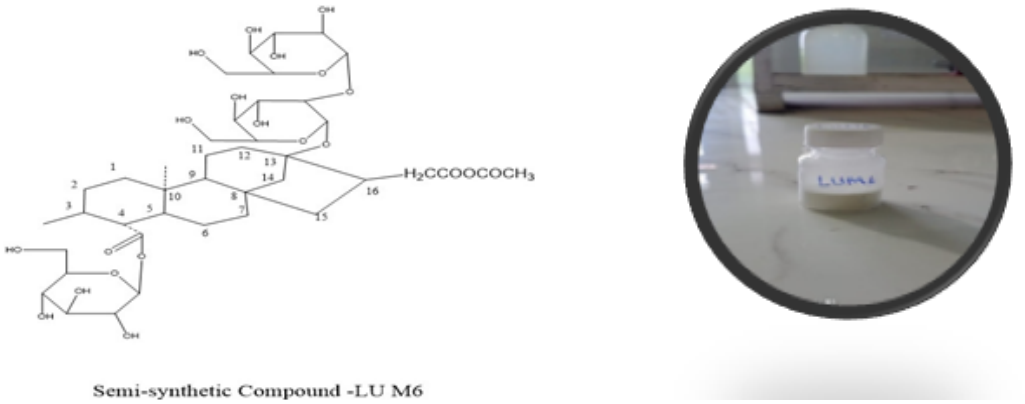


Fig. 35: Semi-synthetic Compound LUM6

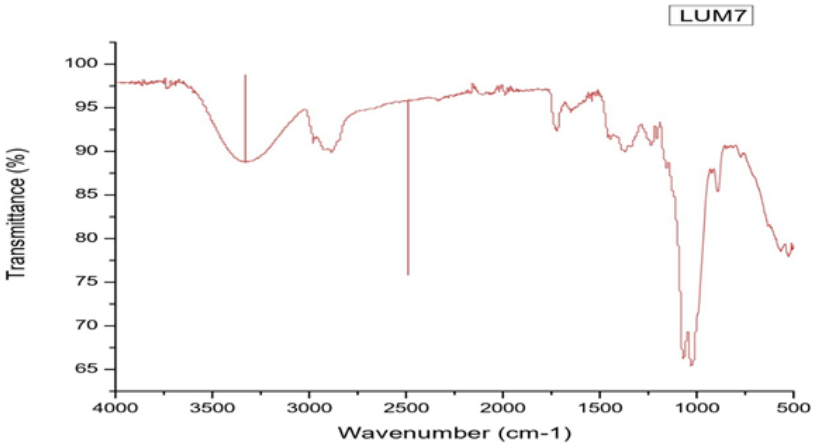


Fig. 36: FTIR Compound LUM7, (CLIF), University of Kerala) Nicolet iS50

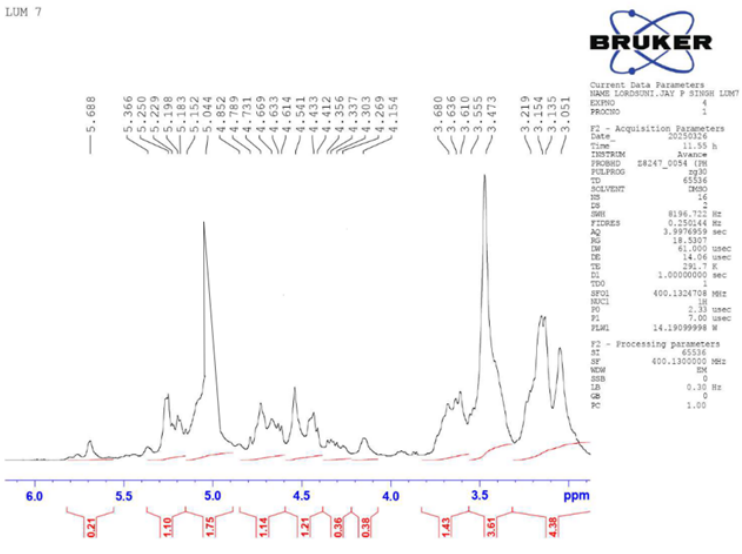


Fig. 37: ^{1}H-NMR Compound LUM7 From, Jamia Hamdard University PROBHD Z108618_0948

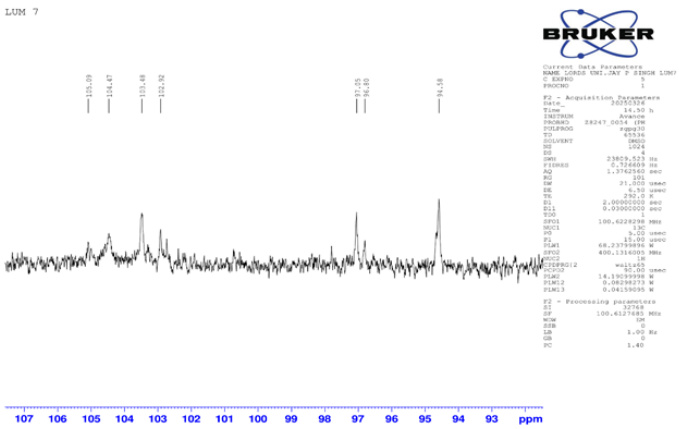


Fig. 38: FTIR Compound LUM7, (CLIF), University of Kerala) Nicolet iS50

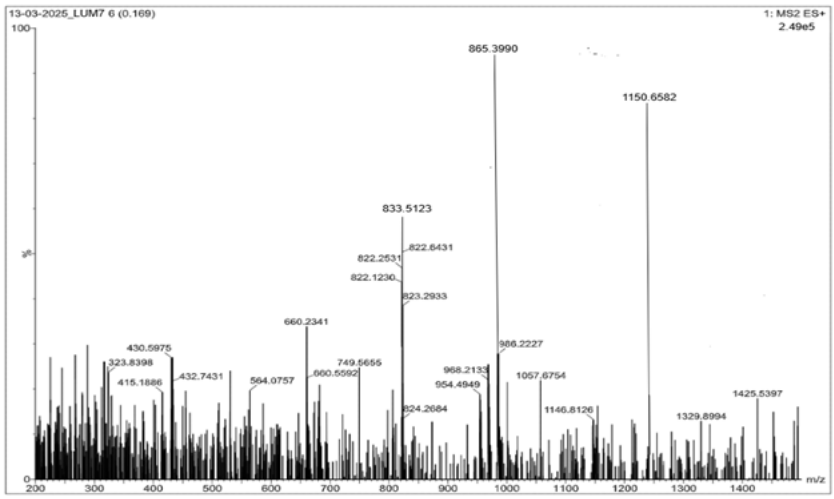


Fig. 39: Mass Spectroscopy Compound LUM7 From, Jamia Hamdard University (M52)

Spectral analysis of compound LUM7

Spectral data:

FTIR Interpretation of LUM7 (Ubeda et al., 2020)

Wavenumber (cm^{-1})	**Functional Group**
Obtained peek 3327cm^{-1}	O-H stretch (very broad)
Obtained peek 2976 cm^{-1}	CH asymmetric stretch
Obtained peek 2880 cm^{-1}	CH symmetric stretch
Obtained peek 1745 cm^{-1}	Ester C=O stretch(s)
Obtained peek 1450 cm^{-1}	CH /CH bending
Obtained peek 1230 cm^{-1}	Ester C-O stretch
Obtained peek 1166 cm^{-1}	C-O-C stretches
Obtained peek 980 cm^{-1}	Pyranose ring vibrations
~1375	CH symmetric bend

1H-NMR Interpretation of LUM7 (Nowacka et al., 2019)

High-Field Region (δ 0.5-2.5 ppm)

Proton	δ (ppm)
H-18	0.91
H-19	1.18
CH CH	1.25
CH CH	Obtained peek 4.15
Ring CH/CH	1.30-2.45

Mid-Field Region (δ2.5-5.5 ppm)

Proton	δ(ppm)
H-8	2.98
H-1'	Obtained peek 4.73
H-1''	Obtained peek 5.25
Sugar CH/CH	Obtained peek 3.21-4.41

Exchangeable Protons (δ4.5-6.5 ppm)

Proton	δ(ppm)
OH (sugar)	Obtained peek 4.8-6.1
OH (steroid)	Obtained peek 5.3-6.4

^{13}C-NMR Interpretation of LUM7 (Mizutani & Tanaka, 2001)

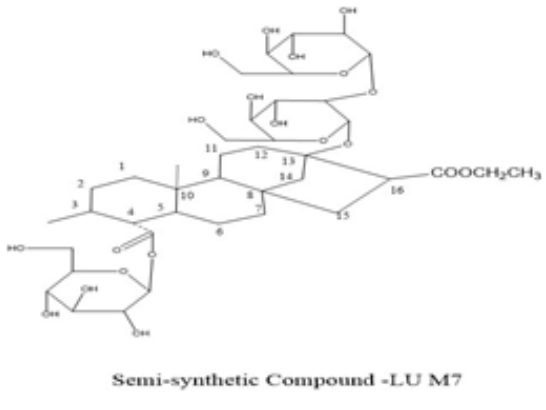
Region (ppm)	Carbon Type
10-25	Methyls
25-45	Aliphatic CH/CH
Obtained peek 60-65	CH OH
70-85	Sugar CH-O
Obtained peek 95-105	Anomeric
Obtained peek 165-175	Ester C=O
Obtained peek 175-180	Glycosidic ester C=O
Obtained peek - CH	60.1
Obtained peek - CH	14.3

891.3940 Unknow Semisynthetic compound LUM1 showed that it is an acetic 2-((4R,8S,11bR)-9-(((2S,3S,5R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2R,5S)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3,11b-dimethyl-4-((((2R,5R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)carbonyl)tetradecahydro-6a,9-methanocyclohepta[a]naphthalen-8-yl)acetic anhydride. Comparison of physical parameter Infrared spectroscopy, Proton NMR, Carbon NMR and Mass Spectroscopy with data evaluation. (Tavarini et al., 2013)

Mass Spectroscopy of LUM7

m/z 865.3990-This is the molecular ion (M+H) + of LUM7, Indicate the molecular weight LUM7 Plus proton. This the most intense peak, representing the LUM7 molecule itself.

Structural Elucidation of Compound LUM7



Semi-synthetic Compound -LU M7

Fig. 40: Semi-synthetic Compound LUM7

Based on interpretation of spectral data, molecular weight 864.93 and M/Z 865.3990 Unknow Semisynthetic compound LUM7 showed that it is a 8-ethyl 4-((2R,5R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl) (4R,8R,11bR)-9-(((2S,3S,5R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2R,5S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3,11b-dimethyltetradecahydro-6a,9-methanocyclohepta[a] naphthalene-4,8-dicarboxylate. Comparison of physical parameter Infrared spectroscopy, Proton NMR, Carbon NMR and Mass Spectroscopy with data evaluation.(Wölwer-Rieck & chemistry, 2012)

CONCLUSION

The study successfully isolated and characterized steviol glycosides from Stevia rebaudiana leaves using solvent extraction techniques, including water, acidic, basic, and alcoholic solvents. The extraction process was optimized with specific temperature controls (75–80°C) and repeated treatments to maximize yield. Rotary evaporation and recrystallization were employed to purify the compounds, yielding stevioside crystals.(Yadav et al., 2012)

Advanced spectroscopic techniques—FTIR, 1H1H-NMR, 13C13C-NMR, and mass spectrometry—were used to elucidate the structures of the isolated compounds (LUM1–LUM7). Key findings include:

1. FTIR confirmed functional groups such as O–H, C=O (ester), and glycosidic bonds.
2. NMR spectra provided detailed proton and carbon environments, identifying anomeric protons and sugar moieties.
3. Mass spectrometry determined molecular weights, with peaks such as *m/z* 805.3421 (stevioside) and *m/z* 891.3940 (LUM6, an anhydride derivative).

The structural elucidation revealed semisynthetic derivatives, including amino (LUM1), imino (LUM2), carboxylic acid (LUM3), formyl (LUM4), carbamoyl (LUM5), anhydride (LUM6), and ethyl ester (LUM7) modifications. These findings highlight the versatility of steviol glycosides for potential applications in food, pharmaceuticals, and nutraceuticals due to their non-caloric sweetness and bioactive properties.

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