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SYNTHESIS AND NMR SPECTRAL CHARACTERIZATION OF OLEANOLIC ACID AND THEIR DERIVATIVES FROM *LANATA CAMARA* ROOTS

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ABSTRACT

Medicinal plants are highly esteemed over the world as a key source for the discovery of new drugs. The isolation or extraction of new drugs or lead molecules from natural products have facilitated a great extend to drug discovery. The study mainly aim to isolate oleanolic acid and their derivatives from the plant sources and the derivatives of the compounds were synthesized and characterized so that in future they can be developed as drug moieties for their physiological activities. The triterpenoid compounds like oleanolic acid, were extracted from the medicinal plant Lanata camara by Vacuum Liquid Chromatography. The fractions obtained during the isolation were pooled on the basis of their TLC profile. The derivatives of oleanolic acid were prepared by acetylation, esterification and amidation reaction at the C-28 position of oleanolic acid. The compounds were characterized using various spectroscopic methods .15 derivatives of oleanolic acid were synthesized and characterization of each of the compound for the identification were carried out using ^{13}C and proton NMR techniques. The NMR profile revealed that all the synthesized compounds were active and can function efficiently for their pharmacological activity

Keywords *– Oleanolic acid, Nuclear Mass Spectroscopy, Pentacyclic triterpenoid, High Performance Liquid Chromatography*

1. INTRODUCTION

Medicinal plants are highly esteemed over the world as a key source for the discovery of new drugs. The isolation or extraction of new drugs or lead molecules from natural products have facilitated a great extend to drug discovery[1,2]. *Lanata camara* belonging to the family verbenaceae is a significant weed used in many parts of the world for the treatment of wide variety of disorders. The plant extracts are used in folk medicine for the treatment of cancer ,chicken pox, asthma, swelling, high blood pressure, rheumatism and malaria etc. Chemical investigation of different parts of *Lanata camara* revealed the presence of number of iridoids, glycosides, naphthoquinone, flavanoids and various triterpenoids like lantanolic and lantic acid. Lanata camara leaf extract exhibited antimicrobial, fungicidal, insecticidal and nematicidal activities. These extracts are also useful in healing excision wounds in experimental animals and play important role in tissue repair process associated with skin injuries[,3,4].

Oleanolic acid glycosides from the several medicinal food stuffs were found to show potent inhibitory activity on increase of serum glucose levels. It was also investigated that the 3-o-glucoronide moiety and the 28-carboxyl group in oleanolic acid exert the

hypoglycemic effect. They do not have any insulin like or insulin releasing activity, but they inhibit the gastric emptying and glucose uptake in the small intestine. The mode of action revealed that the inhibition of gastric emptying was mediated by capsaicin –sensitive sensory nerves and the central nervous system.They also suppress the gastric emptying by stimulating the release or production of dopamine to act through the dopamine-2 receptors which causes the release of prostaglandins.[5,6,7,8]

The major clinical uses of oleanolic acid are anti-ageing property, hair growth stimulant, antimicrobial activity, anti inflammatory, hepato protective and inhibition of tumour promotion[9].

2. MATERIALS AND METHODS

2.1 Processing of Material

2.1.1 Collection of *Lanata camara* roots

The plant species for the extraction of the pentacyclic triterpenoid compound oleanolic acid were collected from the Central Institute of Medicinal and Aromatic Plants(CIMAP), Lucknow and was authenticated by Dr S.C Singh, Scientist, Botany and Pharmacognosy Division ,CIMAP, Lucknow

2.1.2 Extraction of *Lanata camara* Roots

Weighed 700g of *Lanata camara* roots and extracted with hexane at 40^0C and distilled under vacuum which is then extracted using ethyl acetate and distilled under vacuum. The marc after initial extraction with hexane are dissolved in ethanol acetone mixture.

2.2 Isolation and characteriation

2.2.1 Isolation of Oleaolic acid from *Lanata camara* Extract

The isolation was carried out using Ethanol acetone mixture extract by Vacuum Liquid Chromatography (VLC). It consist of a sintered funnel of G1 grade having silica gel –H of TLC grade(without binder) having small particle size about 10µm .20 gm of silica Gel –H was tightly packed in the sintered glass funnel using vacuum and a non-polar solvent like n-hexane was passed through the column for checking the column packing, About 18 gm of ethanol water extract were dissolved in small amount of methanol:chloroform (70:30) mixture.Using a pipette the mixture was spread in the VLC column to form a uniform band. Then completely dry the column under vacuum .The isolation was carried out using different concentrations of the solvent and about 100 ml of each fractions about 89 fractions were collected.The fractions were pooled on the basis of their TLC parameters (solvent system:-SiO2,Chloroform:Methanol in the ratio 9:1 and 9:3 using vanillin –sulphuric acid as the spraying reagent) which resulted in isolation of the oleanolic acid[10,11].

Table 1: Fractionation and pooling of compounds

SOLVENT	FRACTIONS
Hexane: Chloroform(50:50)	1-8
Hexane: Chloroform(30:70)	9-21
Hexane: Chloroform(15:85)	22-28
Chloroform (100%)	29-34
Chloroform: Methanol(99:1)	35-37
Chloroform: Methanol(98.5:1.5)	38-50
Chloroform: Methano (97:3)	51-65
Chloroform: Methanol (96:4)	66-70
Chloroform: Methanol (90:10)	71-79
Chloroform: Methanol (80:20)	80-83
Chloroform: Methanol(50:50)	84-89

2.2.2 Methodology of derivatization of oleanolic acid [12,13, 14]

The major substitution was done on C-3 and C-28 position

1) Acetylation of C-3 hydroxyl group of oleanolic acid
2) Preparation of ester derivatives at C-28 position of 3-O-acetyl oleanolic acid
3) Preparation of amide derivatives at C-28 position of 3-O-acetyl oleanolic acid

1) Acetylation of oleanolic acid

Oleanolic acid (700mg) was dissolved in pyridine(14 ml).To this added 1.5 equivalents of acetic anhydride and the reaction mixture was kept overnight. The reaction was analysed by TLC using hexane: chloroform(7:3) as the solvent system to confirm the completion of reaction. The product was crystallized using crushed ice and was extracted with chloroform to obtain the chloroform extract which was dried .

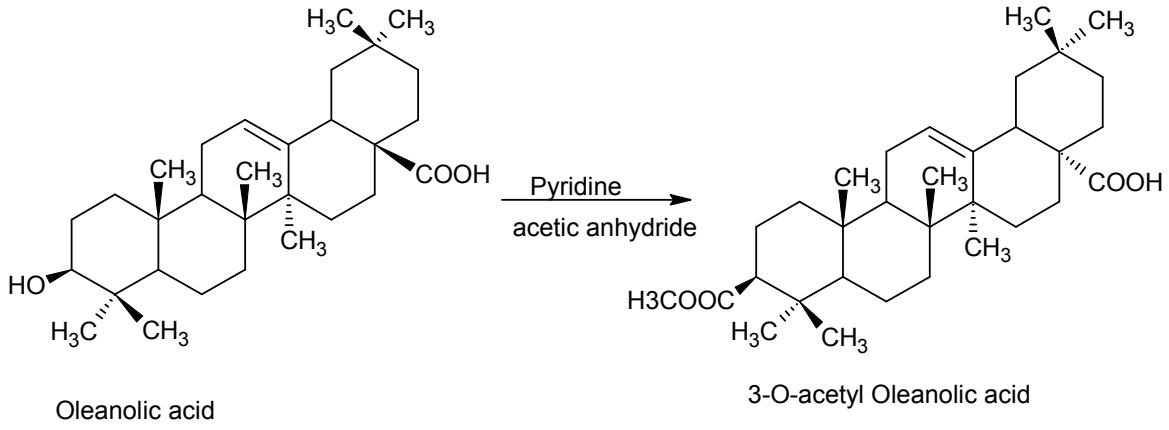

Fig 1: Scheme for the synthesis of acetyl derivative of oleanolic acid

2) Preparation of ester derivatives at C-28 position of 3-O-acetyl oleanolic acid

30 mg of oleanolic acid acetate was dissolved in 10 ml of dry dichloromethane which was flushed with nitrogen and stirred at room temperature for 15 min and then added oxalyl chloride around 1.5 equivalents and flush again with nitrogen and stirred at room temperature for 2-3 hrs. Then the mixture was treated with around 1.5 equivalents of triethyl amine and various alcohols and again reflux for 3-4 hrs. The completion of the reaction was monitored by TLC hexane:chloroform(7:3) as the solvent system by the formation of a non-polar spot. The product was then extracted using ethanol acetone and chloroform.

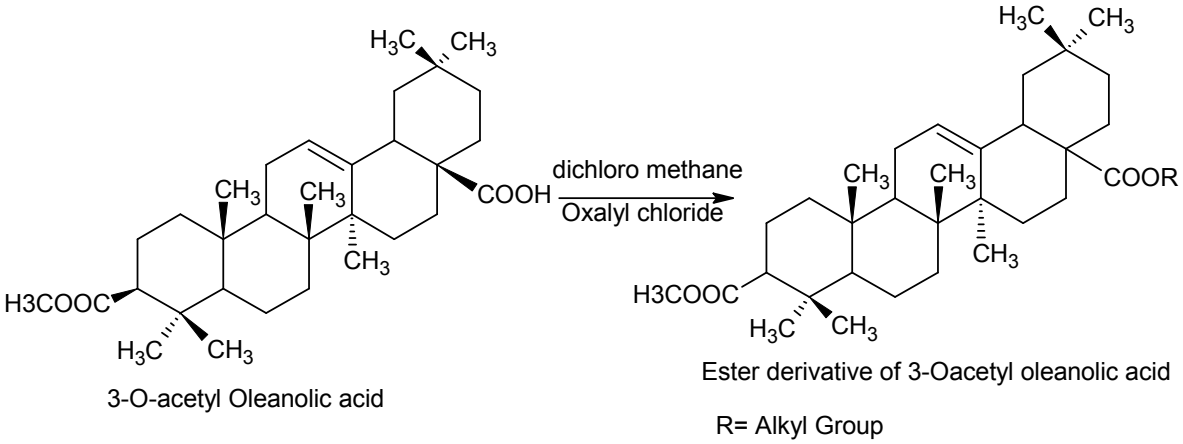

Fig 2: Scheme for the synthesis of ester derivatives of oleanolic acid

3) Preparation of amide derivatives at C-28 position of 3-O-acetyl oleanolic acid

3-O-acetyl oleanolic acid around 50 mg was dissolved in 3 ml of dry Dichloro methane and simultaneously oxalyl chloride around 1.5 equivalents was added and flushed with nitrogen and then stirred at room temperature for3-4 hrs. Then added around 1.2 equivalents of triethyl amine and various amines and the mixture was again refluxed for 3-4 hrs. The completion of the reaction was indicated by TLC using hexane: chloroform (7:3) as the solvent system by the formation of a non-polar spot. It was then extracted with chloroform and dried to get 2-3 spots which was further purified using HPTLC

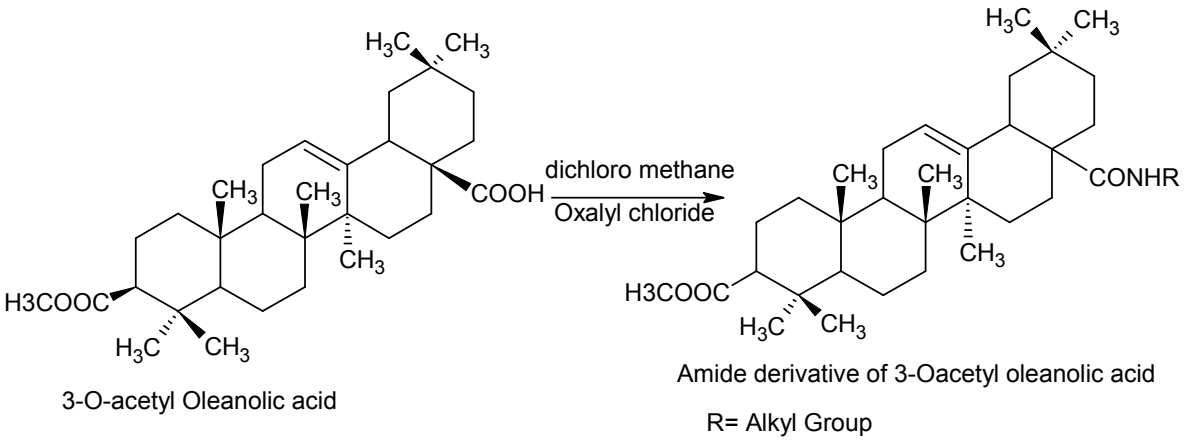

Fig 3: Scheme for the synthesis of amide derivatives of oleanolic acid

3. RESULT AND DISCUSSION

3.1NMR SPECTRAL CHARACTERISATION OF OLEANOLIC ACID DERIVATIVES

3.1.1 Characterization of compound 1

Chemical formula: $C_{30}H_{48}O_3$

m/z ratio :456.36

Melting point :575.17

^{1}H NMR (300MHZ,$CDCl_3$) : δ 0.87,0.96, 0.98, 1.00,1.22, 1.23, 1.27 (3H each,alls,7 x tert.Me) 3.26(1H,d, J= 12.7 Hz,18 β-H) 3.44(1H, brs,3-α-H) 5.48(1H,BRS,12-H)

^{13}C NMR (75 MHZ,CDCl3) : δ 38.5(t, C-1), 23.9(t, C-2), 81.3(d,C-3), 38.1(s, C-4), 55.7(d,C-5) ,18.6(t, C-6), 32.8(t, C-7), 39.7(s, C-8), 47.9(d,C-9), 37.4(s, C-10), 23.3(t, C-11), 123.0(d, C-12), 144.1(s,C-13), 41.9(s,C-14), 28.1(t, C-15), 23.8(t, C-16), 46.9((s, C-17), 41.3(d, C-18), 46.2(t,C-19), 31.1(s, C-20), 34.2(t, C-21), 32.9(t, C-22), 28.4(q, C-23), 17.6(q,C-24), 15.7(q,C-25), 17.1(q, C-26), 26.0(q, C-27), 180.6(COOH, C-28), 33.2(q, C-29), 23.6(q,C-30)

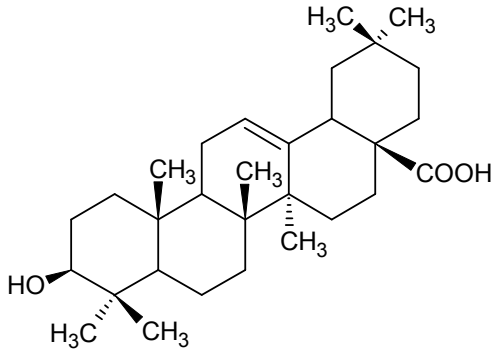

Fig. 4: Structure of Oleanolic acid

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **oleanolic acid**

3.1.2 Characterization of compound 2

Chemical Formula: $C_{32}H_{51}O_4$

Exact Mass:499.4

Mol Weight: 499.7

m/z: 499.38

Solubility: CHCl3

M.P: 580.22

^{1} H NMR (300MHZ,$CDCl_3$) : δ 0.7-1.08 (3H each, s,7 x tert.CH_3), 2.02(3H,s, C-32), 4.47(1H, t, J=8.1 Hz,3 α-H) 5.26 (1H, brs,H-12)

^{13} C NMR (75 MHZ,$CDCl_3$) : δ 38.5(t, C-1), 23.9(t, C-2), 81.3(d,C-3), 38.1(s, C-4), 55.7(d,C-5) ,18.6(t, C-6), 32.8(t, C-7), 39.7(s, C-8), 47.9(d,C-9), 37.4(s, C-10), 23.3(t, C-11), 123.0(d, C-12), 144.1(s,C-13), 41.9(s,C-14), 28.1(t, C-15), 23.8(t, C-16), 46.9((s, C-17), 41.3(d, C-18), 46.2(t,C-19), 31.1(s, C-20), 34.2(t, C-21), 32.9(t, C-22), 28.4(q, C-23), 17.6(q,C-24), 15.7(q,C-25), 17.1 (q, C-26), 26.0(q, C-27), 184.6(COOH, C-28), 33.4(q, C-29), 23.9(q,C-30), 171.3(O-C=O, C-31), 21.6 (q, C-32)

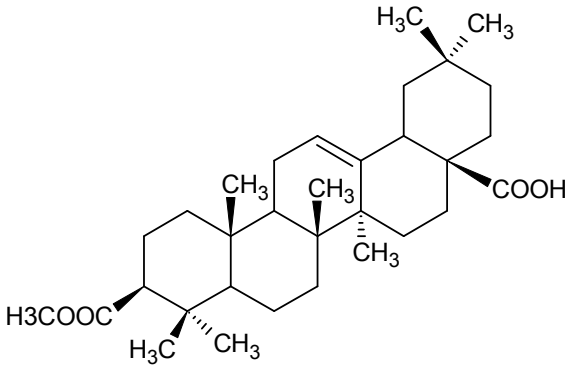

Fig 5: Structure of 3-O-acetyl oleanolic acid

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl oleanolic acid**

3.1.3 Characterization of compound 3

Chemical Formula: $C_{34}H_{54}O_4$

Exact Mass: 526.4

Mol Weight: 526.8

m/z: 526.40

Solubility: $CHCl_3$

M.P: 484.45

^{1} H NMR (300MHZ,$CDCl_3$) : δ 0.73-1.16 (3H each s,7 x tert.CH_3), 2.03 (3H,s, C-32), 4.45(1H, t, J=8.1 Hz,3 α-H) 5.26 (1H, brs,H-12), 4.08(2H, m,H-1'), 1.21(3H,t,J=7.2 Hz, H-2')

^{13} C NMR (75 MHZ,$CDCl_3$) : δ 38.5(CH_2, C-1), 23.9(CH_2, C-2), 81.3(CH,C-3), 38.1(C, C-4), 55.7(CH,C-5) ,18.6(CH_2, C-6), 32.8(CH_2, C-7), 39.8(C, C-8), 48.0(CH,C-9), 37.3(C, C-10), 23.4 (CH_2, C-11), 122.6(CH, C-12), 144.2(C,C-13), 42.1 (C,C-14), 28.1(CH_2, C-15), 23.8 (CH_2, C-16), 46.9((C, C-17), 41.7(CH, C-18), 46.3(CH_2,C-19), 31.0(C, C-20), 34.3 (CH_2, C-21), 33.1 (CH_2, C-22), 28.4 (CH_3, C-23), 17.4 (CH_3,C-24), 15.7(CH_3,C-25), 17.1 (CH_3, C-26), 26.1(CH_3, C-27), 178.0(COOH, C-28), 33.4(CH_3, C-29), 23.9(CH_3,C-30), 171.2(O-C=O, C-31), 21.5 (CH_3, C-32), 60.4(CH_2,C-1'), 14.6(CH_3, C-2')

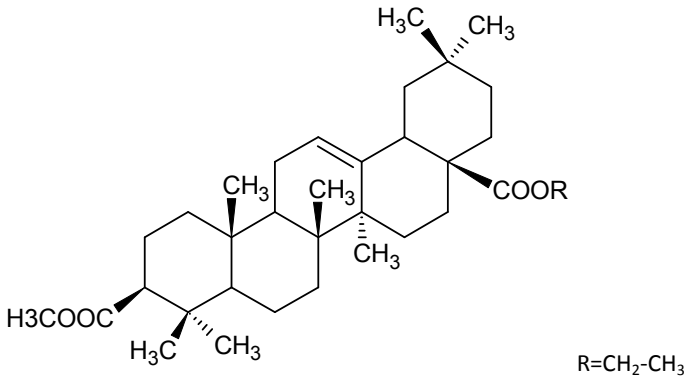

Fig 6: Structure of 3-O- acetylolean-12-en-28-oic acid ethyl ester

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetylolean-12-en-28-oic acid ethyl ester**

3.1.4 Characterization of compound 4

Chemical Formula: $C_{35}H_{56}O_4$

Exact Mass: 540.4

Mol Weight: 540.8

m/z: 540.42

Solubility: $CHCl_3$

M.P: 480.76

^{1} H NMR (300MHZ,$CDCl_3$) : δ 0.72-1.11 (3H each s,7 x tert.CH_3), 2.02 (3H,s, C-32), 4.47(1H, t, J=8.1 Hz,3 α-H) 5.26 (1H, brs,H-12), 4.91 (1H, m,H-1'), 1.17,1.18 (3H each, dd, J=6.3 Hz, H-2' and H-3')

^{13} C NMR (75 MHZ,$CDCl_3$) : δ 38.5(CH_2, C-1), 23.9(CH_2, C-2), 81.3(CH,C-3), 38.1(C, C-4), 55.7(CH,C-5) ,18.6(CH_2, C-6), 32.9(CH_2, C-7), 39.8(C, C-8), 48.0(CH,C-9), 37.3(C, C-10), 23.4 (CH_2, C-11), 122.7(CH, C-12), 144.2(C,C-13), 42.1 (C,C-14), 28.1(CH_2, C-15), 23.9 (CH_2, C-16), 47.2((C, C-17), 41.7(CH, C-18), 46.3(CH_2,C-19), 31.1(C, C-20), 34.3 (CH_2, C-21), 33.1 (CH_2, C-22), 28.4 (CH_3, C-23), 17.4 (CH_3,C-24), 15.7(CH_3,C-25), 17.0 (CH_3, C-26), 26.2(CH_3, C-27), 178.1(COOH, C-28), 33.4(CH_3, C-29), 23.9(CH_3,C-30), 171.3(O-C=O, C-31), 21.5 (CH_3, C-32), 67.3(CH,C-1'), 22.1(CH_3, C-2' and C-3')

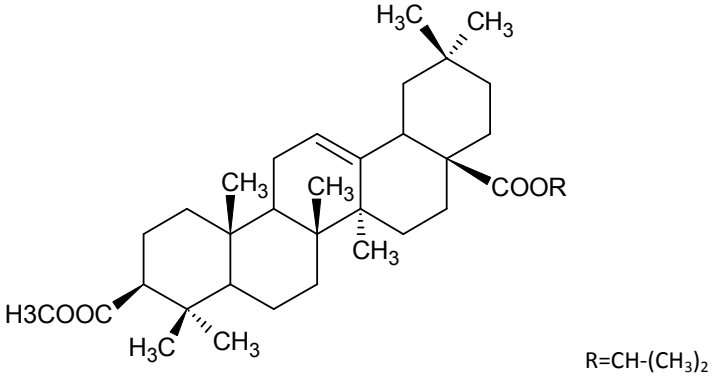

Fig 7: Structure of 3-O-acetyl olean-12-en-28-oic acid isopropyl ester

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl olean-12-en-28-oic acid isopropyl ester**

3.1.5 Characterization of compound 5

Chemical Formula: $C_{36}H_{58}O_4$

Exact Mass: 554.8

Mol Weight: 554.8

m/z: 554.43

Solubility: $CHCl_3$

M.P: 491.95

[1] H NMR (300MHZ,$CDCl_3$) : δ 0.71-1.15 (3H each s,7 x tert.CH_3), 2.08 (3H,s, C-32), 4.47(1H, t, J=8.1 Hz,3 α-H) 5.26 (1H, brs, H-12), 4.06 (2H, d, J=6.6Hz,H-1'), 2.89(1H,m, H-2'), 0.96(6H,d, J=6.6 Hz, H-3' and H-4')

[13] C NMR (75 MHZ,$CDCl_3$) : δ 38.5(CH_2, C-1), 23.8(CH_2, C-2), 81.3(CH,C-3), 38.1(C, C-4), 55.7(CH,C-5) ,18.6(CH_2, C-6), 32.9(CH_2, C-7), 39.8(C, C-8), 48.0(CH,C-9), 37.3(C, C-10), 23.4 (CH_2, C-11), 122.7(CH, C-12), 144.2(C,C-13), 42.1 (C,C-14), 28.1(CH_2, C-15), 23.9 (CH_2, C-16), 47.1((C, C-17), 41.8(CH, C-18), 46.3(CH_2,C-19), 31.1(C, C-20), 34.3 (CH_2, C-21), 33.1 (CH_2, C-22), 28.4 (CH_3, C-23), 17.4 (CH_3,C-24), 15.7(CH_3,C-25), 17.0 (CH_3, C-26), 26.2(CH_3, C-27), 178.1(COOH, C-28), 33.4(CH_3, C-29), 23.9(CH_3,C-30), 171.3(O-C=O, C-31), 21.6 (CH_3, C-32), 70.8 (CH,C-1'), 28.1(CH_3, C-2'), 19.2(CH_3, C-3' and C-4')

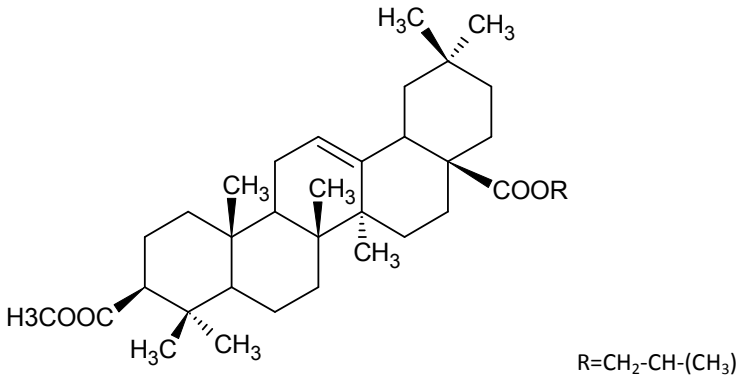

Fig 8: Structure of 3-O-acetylolean-28-oic acid isobutyl ester

Based on the ^1H and ^{13}C NMR data the compound was identified as **3-O-acetylolean-28-oic acid isobutyl ester**

3.1.6 Characterization of compound 6

Chemical Formula: $C_{36}H_{58}O_4$

Exact Mass: 554.4

Mol Weight: 554.8

m/z: 554.43

Solubility: $CHCl_3$

M.P: 509.45

[1] H NMR (300MHZ,$CDCl_3$) : δ 0.71-1.06 (3H each s,7 x tert.CH_3), 1.97 (3H,s, C-32), 4.42 (1H, t, J=7.8 Hz,3 α-H) 5.21 (1H, brs, H-12), 1.34 (9H, s, H-2', H-3' and H-4')

[13] C NMR (75 MHZ,$CDCl_3$) : δ 38.6(CH_2, C-1), 23.8(CH_2, C-2), 81.3(CH,C-3), 38.1(C, C-4), 55.7(CH,C-5) ,18.6(CH_2, C-6), 32.9(CH_2, C-7), 39.9(C, C-8), 48.0(CH,C-9), 37.3(C, C-10), 23.6 (CH_2, C-11), 122.3(CH, C-12), 144.4(C,C-13), 42.3 (C,C-14), 28.1(CH_2, C-15), 23.9 (CH_2, C-16), 47.2((C, C-17), 41.7(CH, C-18), 46.6(CH_2,C-19), 31.1(C, C-20), 34.5 (CH_2, C-21), 33.3 (CH_2, C-22), 28.4 (CH_3, C-23), 17.8 (CH_3,C-24),

15.8(CH_3,C-25), 17.1 (CH_3, C-26), 26.0(CH_3, C-27), 177.1(COOH, C-28), 33.4(CH_3, C-29), 23.9(CH_3,C-30), 171.3(O-C=O, C-31), 21.6 (CH_3, C-32), 84.5 (C,C-1'), 28.4(CH_3, C-2', C-3' and C-4')

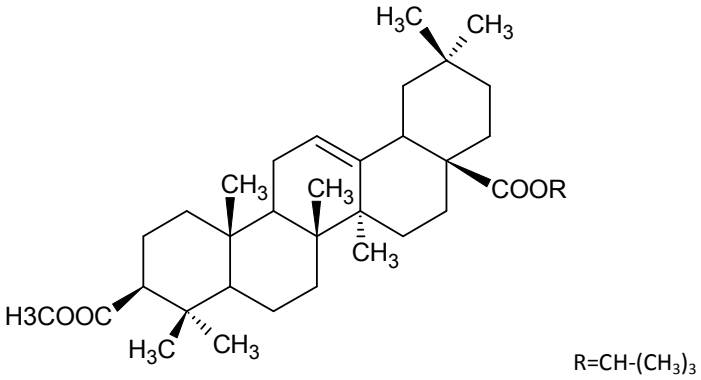

Fig 9: Structure of 3-O-acetyl- olean-12-en 28-oic acid t-butyl ester

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-oic acid t-butyl ester**

3.1.7 Characterization of compound 7

Chemical Formula: $C_{36}H_{58}O_4$

Exact Mass: 554.4

Mol Weight: 554.8

m/z: 554.43

Solubility: $CHCl_3$

M.P: 506.95

^{1} H NMR (300MHZ,$CDCl_3$) : δ 0.83-1.10(3H each s,7 x tert.CH_3), 2.02 (3H,s, C-32), 4.42 (1H, t, J=7.8 Hz,3 α-H) 5.21 (1H, brs, H-12),3.99(2H, t, J=6.3, H-1') 1.61 (2H, m, H-2'),1.41(2H,m,H-3'), 0.90 (3H, m, H-4')

^{13} C NMR (75 MHZ,$CDCl_3$) : δ 38.6(CH_2, C-1), 23.9(CH_2, C-2), 81.3(CH,C-3), 38.1(C, C-4), 55.8(CH,C-5) ,18.6(CH_2, C-6), 32.8(CH_2, C-7), 39.8(C, C-8), 48.0(CH,C-9), 37.4(C, C-10), 23.4(CH_2, C-11), 122.6(CH, C-12), 144.2(C,C-13), 42.1 (C,C-14), 28.1(CH_2, C-15), 23.8 (CH_2, C-16), 47.9((C, C-17), 41.7(CH, C-18), 46.3(CH_2,C-19), 31.1(C, C-20), 34.3(CH_2, C-21), 33.1 (CH_2, C-22), 28.4 (CH_3, C-23), 17.4 (CH_3,C-24), 15.7(CH_3,C-25), 17.0 (CH_3, C-26), 26.2(CH_3, C-27), 178.1(COOH, C-28), 33.4(CH_3, C-29), 24.0(CH_3,C-30), 171.2(O-C=O, C-31), 21.6 (CH_3, C-32), 64.3 (CH_2,C-1'), 30.7(CH_2, C-2'), 19.6(CH_2,C-2' C-3', 14.0 (CH_3, C-4')

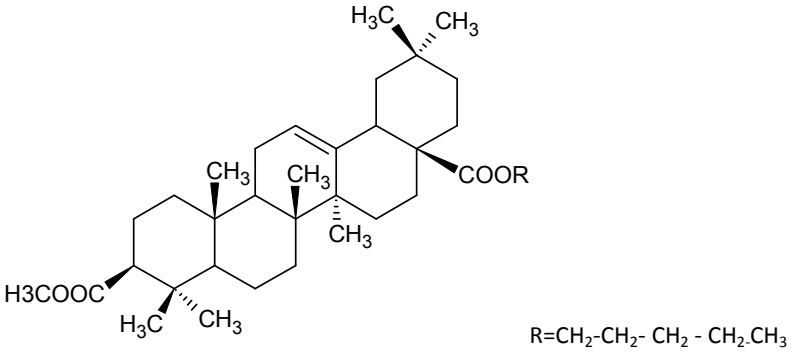

Fig 10: Structure of 3-O-acetyl- olean-12-en 28-oic acid n-butyl ester

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-oic acid n-butyl ester**

3.1.8 Characterization of compound 8

Chemical Formula: $C_{37}H_{60}O_4$

Exact Mass: 568.4

Mol Weight: 568.9

m/z: 568.45

Solubility: $CHCl_3$

[1] H NMR (300MHZ,$CDCl_3$) : δ 0.66-1.08(3H each s,7 x tert.CH_3), 1.97 (3H,s, C-32), 4.41 (1H, t, J=7.8 Hz,3 α-H) 5.19 (1H, brs, H-12), 3.97(2H, t, J=6.3, H-1') 1.57 (2H, m, H-2'), 1.40 (2H,m,H-4'), 0.87 (3H, m, H-5')

[13] C NMR (75 MHZ,$CDCl_3$) : δ 38.6(CH_2, C-1), 23.8(CH_2, C-2), 81.3(CH,C-3), 38.1(C, C-4), 55.7(CH,C-5) ,18.6(CH_2, C-6), 32.8(CH_2, C-7), 39.8(C, C-8), 48.0(CH,C-9), 37.8(C, C-10), 23.4(CH_2, C-11), 122.6(CH, C-12), 144.2(C,C-13), 42.1 (C,C-14), 28.1(CH_2, C-15), 23.9 (CH_2, C-16), 47.0((C, C-17), 41.8(CH, C-18), 46.3(CH_2,C-19), 31.1(C, C-20), 34.3(CH_2, C-21), 33.1 (CH_2, C-22), 28.4 (CH_3, C-23), 17.4 (CH_3,C-24), 15.7(CH_3,C-25), 17.0 (CH_3, C-26), 26.2(CH_3, C-27), 178.1(COOH, C-28), 33.4(CH_3, C-29), 24.0(CH_3,C-30), 171.2(O-C=O, C-31), 21.6 (CH_3, C-32), 63.0 (CH_2,C-1'), 32.8 (CH_2, C-3'), 28.1(CH_2,C-2') ,23.8 (CH_2-C -4'), 15.7(CH_3, C-5')

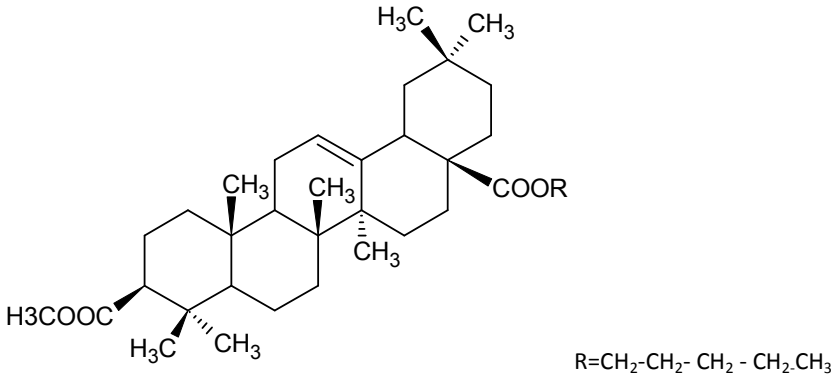

Fig 11: Structure of 3-O-acetyl- olean-12-en 28-oic acid n-amyl ester

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-oic acid n-amyl ester**

3.1.9 Characterization of compound 9

Chemical Formula: $C_{35}H_{57}O_4$

Exact Mass: 539.4

Mol Weight: 539.8

m/z: 539.43

Solubility: $CHCl_3$

[1] H NMR (300MHZ,$CDCl_3$) : δ 0.78-1.18(3H each s,7 x tert.CH_3), 1.98 (3H,s, C-32), 4.4 (1H, t, J=8.4 Hz, 3 α-H), 5.32(1H,brs,H-12) , 5.89 (1H, brs, NH), 3.25 (2H, m, H-1') 1.85 (2H, m, H-2'), 0.70 (3H,brs,H-3'),

[13] C NMR (75 MHZ,CDCl3) : δ 38.5(t, C-1), 23.8 (t, C-2), 81.3 (d,C-3), 38.1(s, C-4), 55.6(d,C-5) ,18.6(t, C-6), 32.7 (t, C-7), 39.8 (s, C-8), 47.9(d,C-9), 37.2(s, C-10), 23.0(t, C-11), 122.9(d, C-12), 145.5(s,C-13), 42.5 (s,C-14), 27.7 (t, C-15), 23.9 (t, C-16), 46.6 (s, C-17), 42.7(d, C-18), 47.2 (t,C-19), 31.1(s, C-20), 34.5(t, C-21), 32.9(t, C-22), 28.4(q, C-23), 17.1(q,C-24), 15.8 (q,C-25), 17.3 (q, C-26), 26.1 (q, C-27), 178.6 (COOH, C-28), 33.4 (q, C-29), 23.9 (q,C-30) 171.4(O-C=O,C-31), 21.7(q, C-32), 41.5 (t,C-1'), 24.2(t,C-2'),11.8(q, C-3')

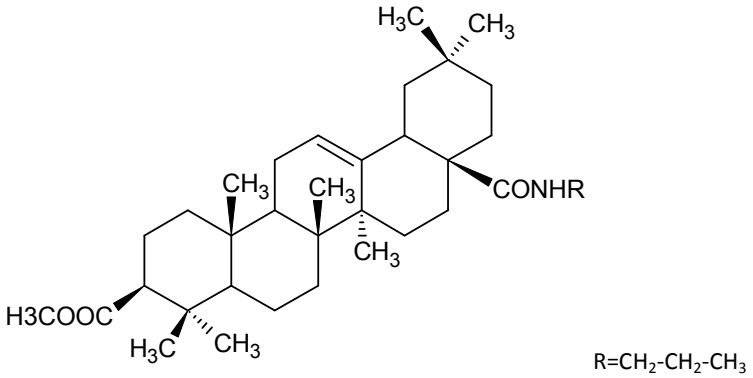

Fig 12: Structure of 3-O-acetyl- olean-12-en 28-oic n-propyl amide

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-oic n-propyl amide**

3.1.10 Characterization of compound 10

Chemical Formula: $C_{40}H_{67}NO_3$

Exact Mass: 609.5

Mol Weight: 610.0

m/z: 609.51

Solubility: $CHCl_3$

^{1} H NMR (300MHZ,$CDCl_3$) : δ 0.84-1.14(3H each s,7 x tert.CH_3), 1.98 (3H,s, C-32), 4.47 (1H, t, J=8.1 Hz, 3 α-H), 5.35(1H,brs,H-12) , 5.90 (1H, brs, NH), 3.30 (2H, m, H-1') 1.90 (2H, m, H-2'), 1.25 (10H,brs,H-3'-7'), 0.75 (3H, brs,H-8')

^{13} C NMR (75 MHZ,CDCl3) : δ 38.5(t, C-1), 24.2 (t, C-2), 81.2 (d,C-3), 38.1(s, C-4), 55.6(d,C-5) ,18.6(t, C-6), 32.7 (t, C-7), 39.8 (s, C-8), 47.9(d,C-9), 37.2(s, C-10), 23.0(t, C-11), 122.9(d, C-12), 145.6(s,C-13), 42.5 (s,C-14), 27.7 (t, C-15), 23.9 (t, C-16), 46.6 (s, C-17), 42.7(d, C-18), 47.2 (t,C-19), 30.1(s, C-20), 32.9 (t, C-21), 32.1(t, C-22), 28.4(q, C-23), 17.1(q,C-24), 15.8 (q,C-25), 17.3 (q, C-26), 26.1 (q, C-27), 178.4 (COOH, C-28), 33.4 (q, C-29), 23.9 (q,C-30) 171.4(O-C=O,C-31), 21.7(q, C-32), 39.8 (t,C-1'), 34.6(t,C-2'),29.4(q, C-3'), 29.5(t,C-4'), 29.6 (t,C-5'), 27.5(t,C-6'), 23.0(t,C-7'), 14.5(q,C-8')

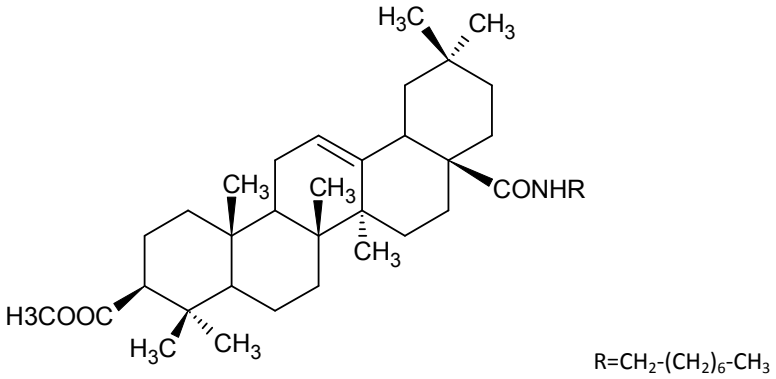

Fig 13: Structure of 3-O-acetyl- olean-12-en 28-octyl amide

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-octyl amide**

3.1.11 Characterization of compound 11

Chemical Formula: $C_{39}H_{57}NO_3$

Exact Mass: 587.4

Mol Weight: 587.9

m/z: 587.43

Solubility: $CHCl_3$

[1] H NMR (300MHZ,$CDCl_3$) : δ 0.59-1.18 (3H each s,7 x tert.CH_3), 1.97 (3H,s, C-32), 4.42 (1H, t, J=8.4 Hz, 3 α-H), 5.23 (1H,brs,H-12) , 6.15 (1H, brs, NH), 4.08, 4.58 (1H each, m, H-1') 7.16-7.30 (5H, m, Ar H)

[13] C NMR (75 MHZ,CDCl3) : δ 38.5(t, C-1), 24.3 (t, C-2), 81.3 (d,C-3), 38.1(s, C-4), 55.7(d,C-5) ,18.6 (t, C-6), 31.0 (t, C-7), 39.9 (s, C-8), 48.0 (d,C-9), 37.4 (s, C-10), 23.8 (t, C-11), 123.2 (d, C-12), 139.0 (s,C-13), 42.9 (s,C-14), 27.8 (t, C-15), 23.9 (t, C-16), 47.2 (s, C-17), 42.6 (d, C-18), 44.0 (t,C-19), 30.0 (s, C-20), 34.6 (t, C-21), 32.9(t, C-22), 28.4(q, C-23), 16.9 (q,C-24), 15.7 (q,C-25), 17.4 (q, C-26), 26.0 (q, C-27), 178.2 (COOH, C-28), 33.3 (q, C-29), 23.9 (q,C-30) 171.0 (O-C=O,C-31), 21.4 (q, C-32), 46.9 (t,C-1'), 145.3 (s,C-1''), 128.9 (d, C-2'', 6''), 128.1(d,C-3'', 5''), 127.6 (t,C-4'')

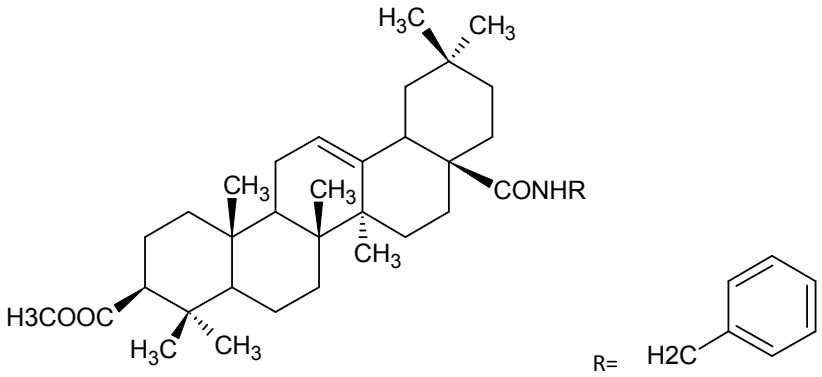

Fig 14: Structure of 3-O-acetyl- olean-12-en 28-benzyl amide

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-benzyl amide**

3.1.12 Characterization of compound 12

Chemical Formula: $C_{40}H_{59}NO_3$

Exact Mass: 601.4

Mol Weight: 601.9

m/z: 601.45

Solubility: $CHCl_3$

[1] H NMR (300MHZ,$CDCl_3$) : δ 0.75-1.21 (3H each s,7 x tert.CH_3), 1.91 (3H,s, C-32), 3.72 (3H, s, C-3'-OCH_3), 4.4 (1H,t,J=8.4 Hz, 3 α H) , 5.23 (1H, brs, H-12), 7.59 (2H , s, H-2, NH') 7.35 (1H, t, J=7.1 Hz, H-5'), 7.7-7.9 (2H, m, H-4', H-6')

[13] C NMR (75 MHZ,CDCl3) : δ 39.5(t, C-1), 24.2 (t, C-2), 82.0 (d,C-3), 39.1(s, C-4), 56.5 (d,C-5) ,19.7 (t, C-6), 34.3 (t, C-7), 40.9 (s, C-8), 49.2 (d,C-9), 38.4 (s, C-10), 25.2 (t, C-11), 123.6 (d, C-12), 140.8 (s,C-13), 43.4 (s,C-14), 29.6 (t, C-15), 25.0 (t, C-16), 47.9 (s, C-17), 43.2 (d, C-18), 47.9 (t,C-19), 32.2 (s, C-20), 34.6 (t, C-21), 31.3 (t, C-22), 29.4 (q, C-23), 18.6 (q,C-24), 15.8 (q,C-25), 18.3 (q, C-26), 27.4 (q, C-27), 181.4 (COOH, C-28), 34.5 (q, C-29), 25.1 (q,C-30) 171.8 (O-C=O,C-31), 22.4 (q, C-32), 160.5 (s,C-1'), 131.4 (d,C-2'), 161.8 (s, C-3'), 115.0 (d,C-4'), 108.7 (d,C-5'), 112.3(d, C-6), 56.8 (q, C-3'-OCH_3)

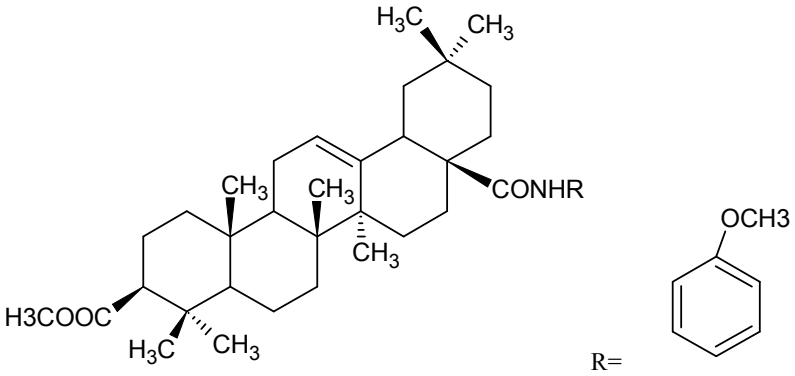

Fig. 15: Structure of 3-O-acetyl- olean-12-en 28-(3)-anisidinamide

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-(3)-anisidinamide**

3.1.13 Characterization of compound 13

Chemical Formula: $C_{39}H_{57}BrNO_3$

Exact Mass: 666.4

Mol Weight: 667.8

m/z: 666.35

Solubility: $CHCl_3$

^{1} H NMR (300MHZ,$CDCl_3$) : δ 0.72-1.18 (3H each s,7 x tert.CH_3), 1.97 (3H,s, C-32), 4.43 (1H, t, J=8.4 Hz, 3 α H) , 5.23 (1H, brs, H-12), 7.2-7.7 (5H, m, Ar-H, NH)

^{13} C NMR (75 MHZ,CDCl3) : δ 38.5(t, C-1), 23.8 (t, C-2), 81.3 (d,C-3), 38.1(s, C-4), 55.7 (d,C-5) ,18.6 (t, C-6), 33.0 (t, C-7), 39.8 (s, C-8), 48.0 (d,C-9), 37.3 (s, C-10), 23.4 (t, C-11), 121.6 (d, C-12), 143.6 (s,C-13), 42.1 (s,C-14), 27.8 (t, C-15), 23.9 (t, C-16), 48.7 (s, C-17), 41.6 (d, C-18), 46.1 (t,C-19), 31.1 (s, C-20), 34.0 (t, C-21), 31.8 (t, C-22), 28.4 (q, C-23), 17.1 (q,C-24), 15.7 (q,C-25), 17.5 (q, C-26), 26.2 (q, C-27), 173.3 (COOH, C-28), 33.4 (q, C-29), 24.0 (q,C-30) 171.5 (O-C=O,C-31), 21.7 (q, C-32), 143.6 (s,C-1'), 123.3 (d,C-2', C-6'), 132.2 (d, C-3', C-5'), 144.0 (s,C-4')

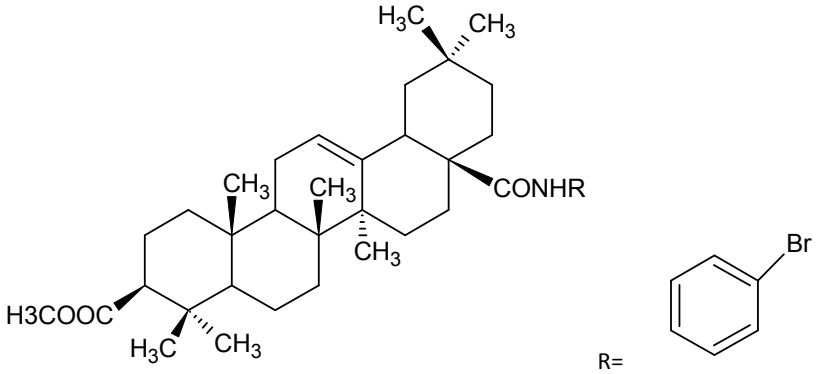

Fig 16: Structure of 3-O-acetyl- olean-12-en 28-(4)-bromo anilamide

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-(4)-bromo anilamide**

3.1.14 Characterization of compound 14

Chemical Formula: $C_{39}H_{56}ClNO_3$

Exact Mass: 621.4

Mol Weight: 622.3

m/z: 621.39

Solubility: $CHCl_3$

[1] H NMR (300MHZ,$CDCl_3$) : δ 0.85-1.25 (3H each s,7 x tert.CH_3), 2.04 (3H,s, C-32), 4.49 (1H, t, J=7.2 Hz, 3 α H) , 5.54 (1H, brs, H-12), 6.60-7.70 (5H, m, Ar-H, NH)

[13] C NMR (75 MHZ,CDCl3) : δ 38.1(t, C-1), 24.1 (t, C-2), 81.3 (d,C-3), 37.3(s, C-4), 55.7 (d,C-5) ,18.5 (t, C-6), 33.0 (t, C-7), 39.8 (s, C-8), 47.9 (d,C-9), 37.2 (s, C-10), 23.4 (t, C-11), 123.4 (d, C-12), 143.6 (s,C-13), 43.1 (s,C-14), 27.9 (t, C-15), 24.0 (t, C-16), 47.8 (s, C-17), 42.2 (d, C-18), 46.1 (t,C-19), 31.1 (s, C-20), 34.6 (t, C-21), 32.3 (t, C-22), 28.4 (q, C-23), 17.1 (q,C-24), 15.8 (q,C-25), 17.6 (q, C-26), 26.2 (q, C-27), 176.9 (COOH, C-28), 33.4 (q, C-29), 23.9.0 (q,C-30) 173.3 (O-C=O,C-31), 21.7 (q, C-32), 139.7 (s,C-1'), 130.4 (d,C-2'), 145.4 (d, C-3'), 123.7 (d,C-4'), 112.5 (d, C-5'), 111.8 (d,C-6')

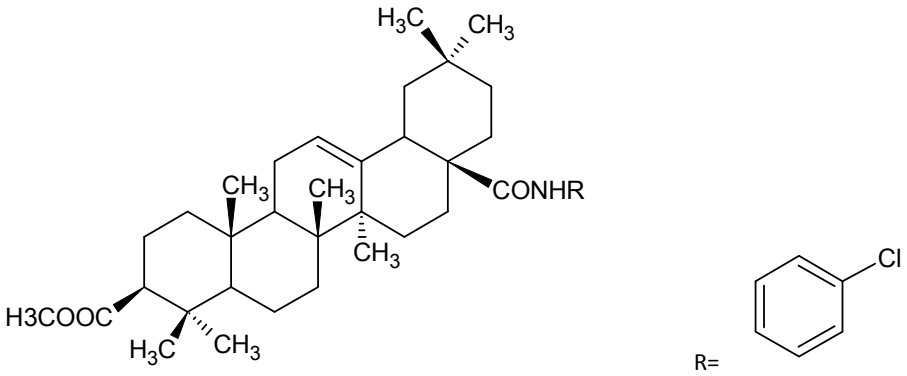

Fig 17: Structure of 3-O-acetyl- olean-12-en 28-(3)-chloro anilamide

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-(3)-chloro anilamide**

3.1.15 Characterization of compound 15

Chemical Formula: $C_{39}H_{56}Cl_2NO_3$

Exact Mass: 656.4

Mol Weight: 656.3

m/z: 656.4

Solubility: $CHCl_3$

[1] H NMR (300MHZ,$CDCl_3$) : δ 0.59-1.18 (3H each s,7 x tert.CH_3), 1.98 (3H,s, C-32), 4.41 (1H, t, J=7.8 Hz, 3 α H) , 5.48 (1H, brs, H-12), 7.60 (1H, s, NH), 7.75 (1H, d, J=2.1 Hz, H-2'), 7.42 (1H,m,H-5'), 7.14 (1H, m, H-6')

[13] C NMR (75 MHZ,CDCl3) : δ 38.6(t, C-1), 24.6 (t, C-2), 81.2 (d,C-3), 38.1 (d, C-4), 55.6 (d,C-5) ,18.5 (t, C-6), 32.7 (t, C-7), 39.8 (s, C-8), 47.8 (d,C-9), 37.2 (s, C-10), 23.9 (t, C-11), 123.9 (d, C-12), 145.4 (s,C-13), 42.6 (s,C-14), 27.7 (t, C-15), 24.1 (t, C-16), 47.8 (s, C-17), 42.9 (d, C-18), 47.0 (t,C-19), 31.1 (s, C-20), 34.5 (t, C-21), 32.6 (t, C-22), 28.4 (q, C-23), 17.0 (q,C-24), 15.8 (q,C-25), 17.3 (q, C-26), 26.1 (q, C-27), 177.2 (COOH, C-28), 33.3 (q, C-29), 23.9.0 (q,C-30) 171.4 (O-C=O,C-31), 21.7 (q, C-32), 127.5 (s,C-1'), 130.7 (d,C-2'), 133.7 (s, C-3'), 137.9 (s,C-4'), 121.8 (d, C-5'), 119.2 (d,C-6')

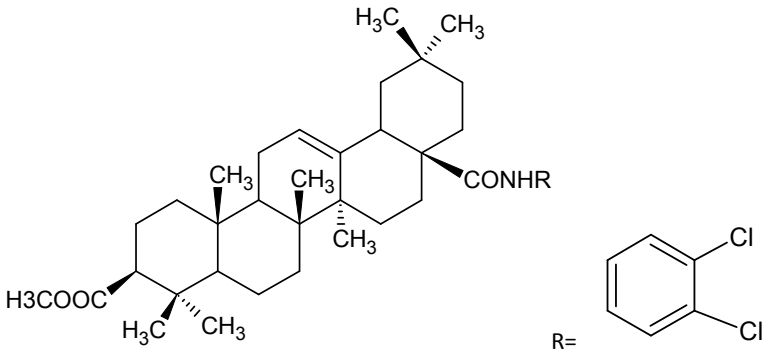

Fig 18: Structure of 3-O-acetyl- olean-12-en 28-(3)-dichloro anilamide

Based on the ^{1}H and ^{13}C NMR data the compound was identified as **3-O-acetyl- olean-12-en 28-(3)-dichloro anilamide.**

4. CONCLUSION

The work deals with the synthesis of oleanolic acid from the root extract of *Lanata camara* and the isolation of various derivative compounds by substitution mainly at theC-3 and C-28 carbon atom. The major reactions undergone were acetylation, amidation and esterification. The isolated oleanolic acid and their derivatives are then characterized using various spectroscopic methods like UV, Mass and NMR spectroscopic methods .Based on the data obtained almost all the derivatives prepared are significant and these compounds can be further studied for their pharmacological activities

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