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STRUCTURAL TRANSFORMATION OF LUPEOL AND EVALUATION THEIR *IN-VITRO* ANTIOXIDANT ACTIVITY

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ABSTRACT: Lupeol (I) is a naturally occurring triterpene that has been isolated from the stem bark of *Crataeva nurvala* in appreciable amount. Due to the presence of a single hydroxyl group and a large, apolar skeleton, lupeol acts as an amphiphile. The two possible sites for chemical modification present in lupeol are ring-A and isopropenyl side chain. We modified it in three naturally occurring analogous derivatives, *i.e.*, 3a, 5a, 5b, 8, 8, 11a-Hexamethyl-1-(2-methyl-oxiranyl)-icosahydro-cyclopenta[a] chrysen-9-ol (II), lupeol acetate (III), and lupenone (IV) by oxidation and acylation process. The structures of the newly synthesized compounds were confirmed by elemental analysis, 1H, 13C NMR, IR, and MS spectroscopy. *In-vitro* antioxidant activity for all the compounds evaluated where ascorbic acid (vitamin C) treated as standard. All the analysis was made with the use of UV-Visible spectrophotometer. Result of the study suggested that all the compounds showed effective efficacy for antioxidant properties in a concentration-dependant manner, although lupeol acetate was found most significant in comparison to standard.

INTRODUCTION: Pentacyclic lupine series triterpenoids such as lupeol or fagarasterol or monogynol B have been extensively studied in the last years as a natural source of therapeutic agents [1]. Lupeol is found in appreciable amounts in common fruit plants such as olive, mango, fig, and strawberry [2]. Lupeol and its derivatives were synthesized and evaluated for their biological activity. They have well recognized for their anti-inflammatory [3], anticancer [4] activities.

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Both *in-vitro* or *in-vivo* and demonstrated a substantial inhibiting effect toward human leukocytic elastase (HLE) [5], HL-60 human leukemia cells, B162F2 murine melanoma cells, p38 proapoptotic mitogen-activated protein kinase (MAPK), and epithelial cells of umbilical melanoma HUVEC.

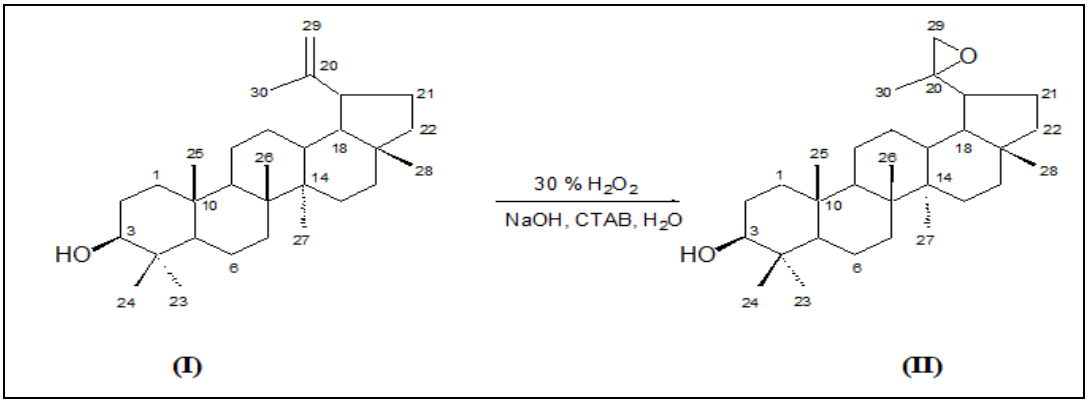
Lupeol derivatives containing functional groups in the ring B displayed a high inhibiting activity toward α-glucosidase and a moderate antibacterial activity [6]. Lupeol ester was found to display cytostatic activity against JB cells [6, 7]. Compounds and were shown to be weak cytostatics [8]. Ester in a concentration range from 5.1 to 9.0 (μg/ml) exhibited a significant inhibiting effect on KB, LNCaP, hTERT-RPE1, and HUVEc cells [9]. Lupane derivative, found in *Camellia japonica* seeds, was

observed to inhibit the induction of Epstein-Barr virus early antigen (EBV-EA). Its effectiveness was comparable to that of the reference inhibitor, β-carotene [10]. Lupeol and its derivatives were reported to possess anti-inflammatory activity due to their effect on prostaglandin biosynthesis [11].Thus it appears that this novel abundantly available 6-6-6-6-5-pentacyclic triterpene having a wide range of biological activities can be used as a template for a particular activity by grafting/crafting different pharmacophores, which can be optimized by chemical modification. Recent investigations also suggest that the free-radical scavenging properties may have great therapeutic importance in free radical-mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis and aging process [12]. In one of our own study lupeol exhibited the antioxidant activity [13]. Therefore, in our present study, we have prepared few novel derivatives of lupeol, and also evaluations were made for their antioxidant efficacy.

MATERIALS AND METHODS:

Experimental:

Extraction and Isolation of Lupeol: Lupeol (1-isopropeny l- 3a, 5a, 5b, 8, 8, 11a -hexamethyl-icosahydro- cyclopenta [a]chrysen-9-ol) is a major constituent of the bark of *Crataeva nurvala*. It was isolated by the extracting the bark with petroleum ether. It was isolated in Petroleum ether: Benzene (1:1) solvent system and crystallized with methanol and Yellowish-white crystals were obtained. M.P. was observed 220 °C. Its molecular formula was calculated as $C_{30}H_{50}O$.



SCHEME 1

Characterization Data of The Compound I: MS (m/z) 427 (M+H), 426 (M+). Molecular formula calculated as $C_{30}H_{50}O$. IR (KBr, Cm^{-1}) 3635(OH stretching), 1650 (>C=C< stretching), 1390 and 1365 (C-H stretching of >CMe2 group). ^{1}HNMR (δ ppm, $CDCl_3$): 4.68 (s, 1H, C-29), 4.55 (s, 1H, C-29), 3.19 (t, 1H, C-3), 2.35 (m, 2H, C-21), 1.68 (s, 3H, C-30), 0.97 (s, 3H, C-23), 0.92 (s, 3H, C-28), 0.84 (s, 3H, C-26), 0.81 (s, 3H, C-24), 0.77 (s, 3H, C-25), 0.75 (s, 3H, C-27), 1.24-1.74 (remaining 23 protons). ^{13}CNMR (δ ppm, $CDCl_3$): 38.70 (C-1), 27.45 (C-2), 79.41 (C-3), 39.00 (C-4), 55.23 (C-5), 18.37 (C-6), 34.38 (C-7), 41.02 (C-8), 50.45 (C-9), 37.22 (C-10), 21.03 (C-11), 24.98 (C-12), 38.10 (C-13), 43.08 (C-14), 27.50 (C-15), 35.67 (C-16), 42.86 (C-17), 48.30 (C-18), 48.02 (C-19), 151.33 (C-20), 29.05 (C-21), 40.06 (C-22C-26), 28.00 (C-23), 15.50 (C-24), 16.02 (C-25), 16.15 (), 14.56 (C-27), 18.01 (C-28), 109.74 (C-29), 19.37 (C-30).

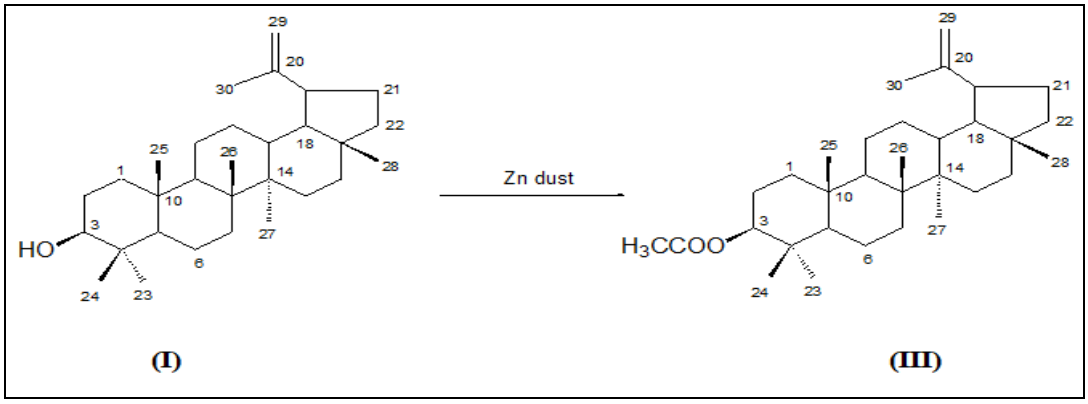
Synthesis of 3a, 5a, 5b, 8, 8, 11a -Hexamethyl-1-(2-Methyl-Oxiranyl) -Icosahydro-Cyclopenta [A] Chrysen-9-Ol (II): Lupeol with (1.0 mmol), aqueous 30% hydrogen peroxide (2.0 mmol), cetyletrimethylammmonium bromide (0.05 mmol), water (5.0 ml), sodium hydroxide (1.0 mmol) were added into a 50 ml. round bottom flask and the reaction mixture was allowed to stir magnetically at 60 °C for 6 h.

Progress of the reaction was monitored by TLC (petroleum ether: ethyl acetate, 4:1), and visualization was accomplished in an iodine chamber. After completion of the reaction, the solid obtained was collected by filtration and washed with warm water. The crude product so obtained, was purified by column chromatography followed by crystallization with ethanol to afford pure product II.

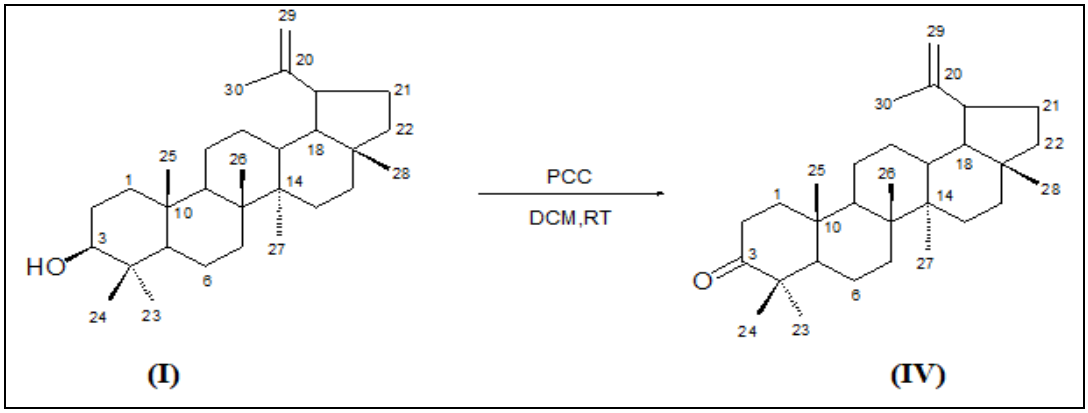
Characterization Data of the Compound II: IR (Kbr, Cm^{-1}) Y_{max}: 3290 (OH stretching), 1390 and 1365 (C-H stretching of >CMe2 group), 1195 (–C–O–C– stretching), etc. ^1H NMR (δ, $CDCl_3$, 300.15 MHz) : 2.46 (s, 2H, C-29), 3.19 (t, 1H, C-3), 2.35 (m, 2H, C-21), 1.65 (s, 3H, C-30), 0.96 (s, 3H, C-23), 0.94 (s, 3H, C-28), 0.84 (s, 3H, C-26), 0.82 (s, 3H, C-24), 0.78 (s, 3H, C-25), 0.76 (s, 3H, C-27), 1.20-1.61 (remaining 23 protons). 13C NMR (δ, $CDCl_3$, 75.47 MHz) : 38.70 (C-1), 27.45 (C-2), 79.41 (C-3), 39.00 (C-4), 55.23 (C-5), 18.31 (C-6), 33.77 (C-7), 40.33 (C-8), 49.93 (C-9), 37.54 (C-10), 20.43 (C-11), 24.63 (C-12), 38.20 (C-13), 42.51 (C-14), 27.49 (C-15), 35.09 (C-16), 42.51 (C-17), 47.80 (C-18), 47.49 (C-19), 52.49 (C-20), 29.35 (C-21), 40.06 (C-22), 28.00 (C-23), 15.48 (C-24), 17.50 (C-25), 15.63 (C-26), 14.88 (C-27), 18.31 (C-28), 58.29 (C-29), 20.43 (C-30). Light yellow crystals; M.P. 178 °C; Yield: 63%.

Synthesis of Lupeol Acetate (III): Lupeol (100 mg) and Acyl chloride (300 mg) were taken in a round bottom flask. To this, zinc dust (0.08 g) was added and stirred for a five h. The progress of the reaction was monitored by TLC.

After completion of the reaction, ether was added. The organic layer was washed successively with saturated $NaHCO_3$ and water and dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by silica gel column chromatography to afford pure lupeol acetate.



SCHEME 2



SCHEME 3

Characterization Data of the Compound III: MS (m/z): 491 (M+ + Na), 468 (M+), 453, 423, 410 (base peak), 391, 385, 327, 281, 175, 161, 147, 135, 121, 107. Molecular formula calculated as $C_{32}H_{52}O_2$. IR (KBr, Cm^{-1}) 1733 (>C=O stretching), 1652 (C=C stretching), 1385, 1370 (gem dimethyl group) and 1050 (C-O stretching). ^1HNMR (δ ppm, $CDCl_3$): 4.44 (dd, 1H, C-3), 1.64 (s, 3H, C-30), 2.36 (m, 2H, C-21), 1.04 (s, 3H, C-23), 0.78 (s, 3H, C-24), 0.87 (s, 3H, C-25), 0.93 (s, 3H, C-26), 0.84 (s, 3H, C-27), 0.96 (s, 3H, C-28), 4.56 (br, s, 1H, C-29), 4.68 (br, s, 1H, C-29), 2.04 (s, 3H, -$OCOCH_3$), 1.25-1.68 (remaining 23 protons). 13C NMR (δ ppm, $CDCl_3$): 38.33 (C^{-1}), 27.38 (C-2), 80.94, C-3), 37.76 (C-4), 55.33 (C-5), 18.16 (C-6), 34.15 (C-7), 39.96 (C-8), 50.29 (C-9), 37.03 (C-10), 21.32 (C-11), 25.03 (C-12), 37.98 (C-13), 42.96 (C-14), 27.91 (C-15), 35.52 (C-16), 42.77

(C-17), 47.97 (C-18), 48.23 (C-19), 150.96 (C-20), 29.78 (C-21), 40.79 (C-22), 27.38 (C-23), 14.46 (C-24), 17.96 (C-25), 16.47 (C-26), 16.15 (C-27), 19.25 (C-28), 109.33 (C-29), 20.89 (C-30), 171.03 (-$OCOCH_3$ at C-3), 23.67 (-$OCOCH_3$ at C-3).

Synthesis of Lupenone (IV): Lupeol I (100 mg) dissolved in DCM (8 ml) was stirred at room temperature with PCC (64 mg) for 28 h. After completion of reaction (TLC), work up as in the above case gave 170 mg of pure lupenone derivative.

Characterization Data of the Compound IV: Mass (m/z) 424 [M+], 409, 381, 218, 205, 204, 203, 189, *etc*. Molecular formula calculated as $C_{30}H_{48}O$. IR (vmax) cm^{-1}(KBr) 2990, 2850, 1735, 1640, 1385, 1360, 1310, 1290, 1270, 980, 970, 880 and 830. 1H NMR [300 MHz, δ (ppm)] ($CDCl_3$) 4.69 (1H, d), 4.57 (1H, d), 2.17 (2H, m), 1.68 (3H, s), 1.58-1.13 (23H, m), 1.03 (3H, s), 1.00 (3H, s), 0.96 (3H, s), 0.94 (6H, s) and 0.76 (3H, s).

Evaluation of Antioxidant Activity: The antioxidant activity of all the prepared compounds was determined by different *in-vitro* methods such as the DPPH scavenging assay, Nitric oxide radical assay, Hydroxyl radical scavenging activity and Hydrogen peroxide scavenging activity. Concentrations of standard and different compounds were used as follows:

Standard: Ascorbic acid (Vitamin C) at 5, 10, 15, 20 µg/ml.

Compound II: 3a, 5a, 5b, 8, 8, 11a -Hexamethyl-1-(2-methyl-oxiranyl) -icosahydro-cyclopenta [a] chrysen-9-ol at 5, 10, 15, 20 µg/ml.

Compound III: Lupeol acetate at 5, 10, 15, 20 µg/ml.

Compound IV: Lupenone at 5, 10, 15, 20 µg/ml.

DPPH Scavenging Activity: DPPH method [14] of Shirwaiker *et al*. was used to evaluate the free radical scavenging capacity of the compounds. Different concentration (5, 10, 15, 20 µg/ml) of compounds prepared in methanol were mixed with 0.1 mM DPPH solution dissolved in 95% methanol. After half an hour the absorbance of the solution was measured at 517 nm using spectrophotometer.

Nitric Oxide Radical Activity: Garret *et al*. Method [15] was employ to conduct nitric oxide radical activity. Different concentration (5, 10, 15, 20 µg/ml) of compounds were mixed with 5 mM sodium nitro prusside dissolved in phosphate buffer saline. This solution was incubated at 250 °C for 150 min and further reacted with Griess reagent. Absorbance of the solution was read at 546 nm. Ascorbic acid (Vitamin C) at 5, 10, 15, 20 µg/ml concentrations were as standard.

Hydroxyl radical scavenging activity: Salicylic acid method suggested by Smirnoff and Cumbes [16] was used. 1 ml of solution of different concentration of all the compounds was mixed with 1 ml of 9 mmol/l salicylic acid, 1ml of 9 mmol/l ferrous sulphate and 1ml of 9 mmol/l hydrogen peroxide. This mixture was incubated at 37 °C for 60 min and absorbance further measured at 510nm using spectrophotometer.

Hydrogen Peroxide Scavenging Activity: Scavenge hydrogen peroxide activity of all the compounds was determined by following the method [17] of Ruch *et al*. 5, 10, 15, 20 µg/ml concentrations of individual compounds was mixed with 0.6 ml (40 mM) hydrogen peroxide solution. Absorbance was measured at 230 nm.

All the experiments were performed thrice. An average reading was taken for the calculation by following the formula for scavenging activity.

$$\% \text{ Scavenging activity} = A_0 - A1/A_0 \times 100$$

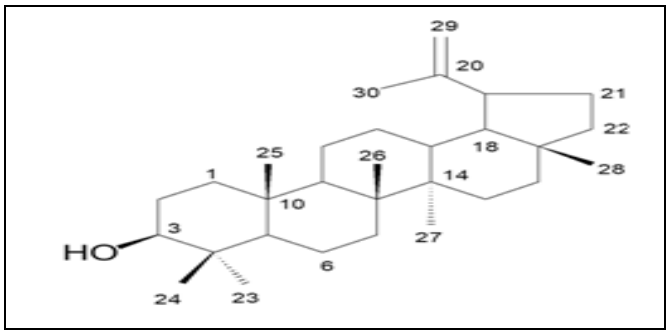
Where A_o = Absorbance of control A1 = Absorbance of sample

RESULTS AND DISCUSSION:

Characterization of Lupeol: The structure of the compound was confirmed by spectral studies. Lupeol gave a +ve Libberman-Burchard test and Noller's reagents and thus confirmed its triterpenoid nature. The mass spectrum of the compound showed molecular ion peak at m/z 426 (M+). In the proton NMR spectrum the presence of six tertiary methyl groups were observed at 0.75 (s, 3H, C-27), 0.77 (s, 3H, C-25), 0.81 (s, 3H, C-24), 0.84 (s, 3H, C-26), 0.92 (s, 3H, C-28) and 0.97 (s, 3H, C-23). The methyl group attached to olefinic carbon was observed as a singlet at 1.68 for three protons.

Chemically different vinylic protons attached at C-29 were observed as a pair of broad singlets at 4.68 and 4.55 for one proton each. The proton present at C-3 position was observed at 3.19 as a triplet. A multiplet at 2.35 was assigned for two protons present at the C-21 position in pentacyclic ring. A complex pattern observed in the region from 1.24 to 1.74 as a multiplet was assign to the remaining twenty-three protons of the molecule.

The 13C NMR spectrum (δ ppm, $CDCl_3$) showed characteristic absorptions for olefinic carbon atoms at 109.74 and 151.33. The attachment of hydroxyl group at C-3 position was confirmed by a signal observed at 79.41. These spectral data are in good agreement with those reported for lupeol in the literature [16]. On the basis of these observations, compound was identified as lupeol.



LUPEOL

The present investigation deals with the synthesis of a novel derivative of lupeol. The syntheses of natural lupeol analogues II-IV were accomplished by subjecting various chemical reactions.

Chemical Modifications: In this article, we describe the work on the isopropenyl Side chain and Alcohalic functionality of Lupeol. Synthesis of compound (II) has been carried out by its oxidation by aqueous 30% hydrogen peroxide (1.2 mmol) and CTAB. We have also synthesized Lupeol acetate (III) via acetylation of Lupeol in the presence of Zn dust. Lupeol has also been modified in Lupenone by its oxidation through Pyridinium Chlorochromate (PCC). The isolated compound was characterized on the basis of their spectral (IR, 1H, and 13C NMR.) studies.

Characterization of 3a, 5a, 5b, 8, 8, 11a -Hexamethyl -1-(2-Methyl-Oxiranyl) -Icosahydro-Cyclopenta[A] Chrysen-9-Ol (II): The IR spectrum (KBr, cm^{-1}) showed strong absorption at 3290 cm^{-1} suggested the presence of hydroxyl group. Sharp absorptions observed at 1390 and 1365 are characteristic of bending vibrations of the gem dimethyl group (>CMe2). The absorption band appeared at 1195 cm^{-1} due to –C–O–C– linkage with the absence of the characteristic absorption at 1650(>C=C<) confirmed the formation of the product.

In the proton NMR (δ ppm, $CDCl_3$) the presence of six tertiary methyl groups were observed at 0.76 (s, 3H, C-27), 0.78 (s, 3H, C-25), 0.82 (s, 3H, C-24), 0.84 (s, 3H, C-26), 0.94 (s, 3H, C-28) and 0.96 (s, 3H, C-23). The methyl group attached to oxirane ring was observed as a singlet at 1.26 for three protons. The proton present at C-3 position was observed at 3.19 as a triplet. A multiplet at 2.35 was assigned for two protons present at C-21 position in pentacyclic ring. The remaining twenty-three protons were observed in the region from 1.20 to 1.61 as a complicated pattern. The signal appeared at 2.46 for two protons, confirmed the presence of oxirane ring instead of 4.68 and 4.55 (due to vinylic protons attached at C-29), further confirmed the formation of the desired product.

The 13C NMR spectrum (δ ppm, $CDCl_3$) showed characteristic absorptions for carbon atoms of oxirane ring at 58.2 and 52.4 with the absence of signals at 109.74 and 151.33 due to olefinic carbons. The attachment of hydroxyl group at C-3 position was confirmed by a signal observed at 79.41.

Other absorptions observed at 38.77 (C-1), 27.45 (C-2), 39.00 (C-4), 55.23 (C-5), 18.31 (C-6), 33.77 (C-7), 40.33 (C-8), 49.93 (C-9), 37.54 (C-10), 20.43 (C-11), 24.63 (C-12), 38.20 (C-13), 42.51 (C-14), 27.49 (C-15), 35.09 (C-16), 42.51 (C-17), 47.80 (C-18), 47.49 (C-19), 29.35 (C-21), 40.06 (C-22), 28.00 (C-23), 15.48 (C-24), 17.50 (C-25), 15.63 (C-26), 14.88 (C-27), 18.31 (C-28) and 20.43 (C-30) and their assignment has been done accordingly as shown in parentheses. On the basis of IR, 1H NMR and 13C NMR spectra the structure of compound was confirmed as 3a, 5a, 5b, 8, 8, 11a -Hexamethyl-1-(2-methyl-oxiranyl)-icosahydro-cyclopenta [a] chrysen-9-ol.

Characterization of Lupeol Acetate (III): The FAB mass spectrum of the compound showed

significant signals at m/z 491 (M+ + Na), 468 (M+), 453, 423, 410, 391, 385, 327, 281, 175, 161, 147, 135, 121, 107 *etc*. On the basis of molecular ion peak the molecular formula for this compound was confirmed as $C_{32}H_{52}O_2$. The IR spectrum (KBr, Cm^{-1}) of compound III showed sharp absorption at 1733, which confirmed the presence of an acetoxyl group in the molecule.

The presence of >C=C< was confirmed by the characteristic absorption at 1652. Absorption at 1050 confirmed the presence of –C–O–C linkage. The other prominent absorptions at 1385 and 1370, were characterized for the presence of gem dimethyl group (>CMe2) in the title compound. The proton NMR spectrum (δppm, $CDCl_3$) showed sharp singlets at 0.78, 0.84, 0.87, 0.93, 0.96 and 1.04, indicating six methyl groups at C-24 C-27, C-25, C-26, C-28 and C-23 positions, respectively.

The three protons of a methyl group at the C-30 position were confirmed by the presence of a sharp singlet at 1.64. The presence of three protons of the acetyl group at 2.04 as a sharp singlet confirmed its position at C-3. The proton present at carbon atom C-3 was observed at 4.44 as a double doublet. Two protons of the pentacyclic ring at C–21 position were confirmed by a multiplet at 2.36.

A pair of broad singlets at 4.56 and 4.68 was assigned to the vinylic protons attached at C-29. The presence of the remaining twenty-three protons was calculated in the region from 1.24 to 1.70. In the 13C NMR spectrum (δppm, $CDCl_3$), absorptions observed at 27.38 (C-23), 14.46 (C–24), 17.96 (C-25), 16.47 (C-26), 16.15 (C-27) and 19.25 (C-28) confirmed the presence of six methyl groups.

The signals observed at 109.33 and 150.96 were assigned for carbon-carbon double bond at C-29 and C-20 carbon atoms, respectively. The absorption for methyl group at C-30, which is attached to an olefinic carbon atom, appeared at 20.89.

The presence of absorption at 80.94 showed the presence of an acetoxy group attached at C-3 position. The absorptions appearing at 171.03 and 23.67 clearly indicated the presence of acetoxyl (-$OCOCH_3$) group. The values of other carbon atoms in compound were established as 38.33 (C–1), 27.38 (C–2), 37.76 (C–4), 55.33 (C–5), 18.16 (C–6), 34.15 (C–7), 39.96 (C–8), 50.29 (C–9), 37.03 (C–10), 21.32 (C–11), 25.03 (C–12), 37.98 (C–13), 42.96 (C–14), 27.91 (C–15), 35.52 (C–16),42.77 (C–17), 47.97 (C–18), 48.23 (C–19), 29.78 (C–21) and 40.79 (C–22).

Characterization of Lupenone (IV): In its IR spectrum important peaks were observed at 1735 (C=O stretching), 1640, and 880 (C=CH_2 stretching), 1385, 1360, 1310, 1290, and 1270 cm^{-1} (characteristic of lupane series). The 1H NMR spectrum showed a pair of doublets at δ 4.57 and 4.69 correspondings to two vinylidene protons of isopropenyl side chain while the singlet at δ 1.68 could be assigned to the olefinic methyl group in the side chain.

A multiplet at δ 2.17 was observed due to C-2 methylene protons in the vicinity of carbonyl function. The six methyl groups located on saturated carbons resonated as singlets at δ 0.76 (3H), 0.94 (6H), 0.96 (3H), 1.00 (3H) and 1.03 (3H). The methylene and methine protons appeared as multiplets in the region δ 1.13-1.58.

Its mass spectrum exhibited a molecular ion peak at m/z 424 [M+] corresponding to its molecular formula $C_{30}H_{48}O$. The other peaks were observed at m/z 409 [M+–15], 381 [M+– 43], 218 [$C_{16}H_{26}$], 205 [$C_{14}H_{21}O$, base peak] and 189 [$C_{14}H_{21}$]. The above spectral data were in close agreement to those reported18 for lupenone, m.p. 167 °C.

Evaluation of *In-vitro* Antioxidant study:

DPPH Scavenging Activity: DPPH scavenging ability of compounds II, III, and IV is shown in **Fig. 1.** which illustrated that the percentages of inhibitions were increased with increasing concentrations of the compounds.

The highest IC_{50} value for DPPH scavenging activity of standard was found 83.99 for 20 µg/ml. in comparison to standard the most significant result was observed for compound III for the same concentration.

The least effective efficacy was shown by compound II *i.e.* 33.91, 39.57, 48.60 and 61.41 for concentrations 5, 10, 15 and 20 µg/ml, respectively.

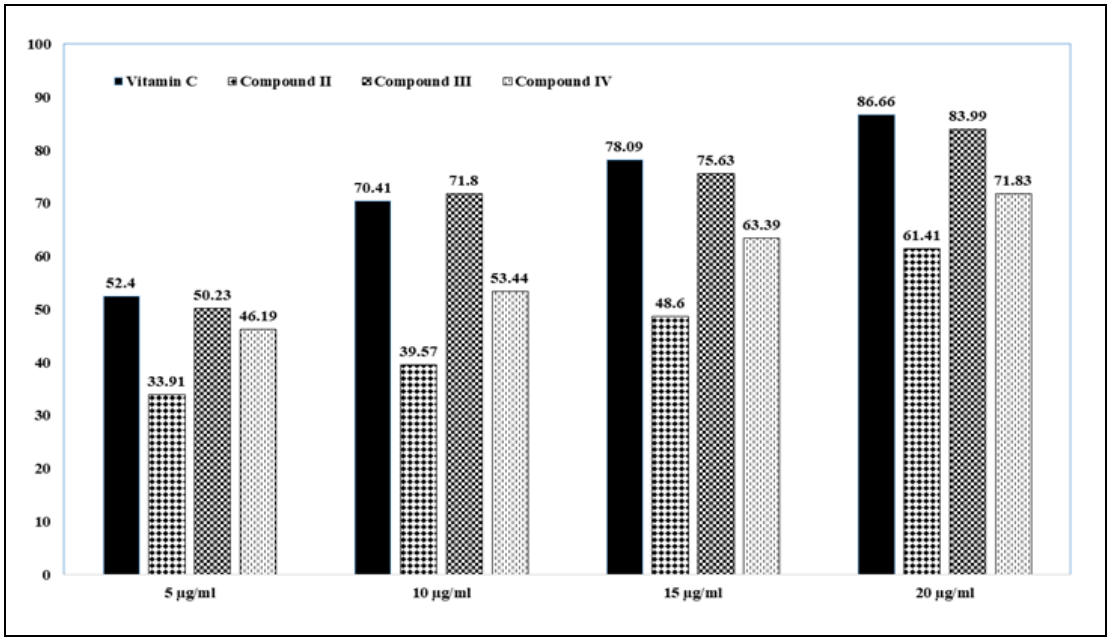


FIG. 1: DPPH SCAVENGING ACTIVITY

Nitric Oxide Radical Activity: The percentages of inhibitions were increased with increasing concentrations. The IC_{50} value for scavenging of nitric oxide was found lowest 28.27 for compound II at 5 µg/ml while highest 62.40 for compound III at 20 µg/ml, while the IC_{50} value for the standard was observed 40.09 and 60.93 for 5 µg/ml and 20 µg/ml respectively **Fig. 2.**

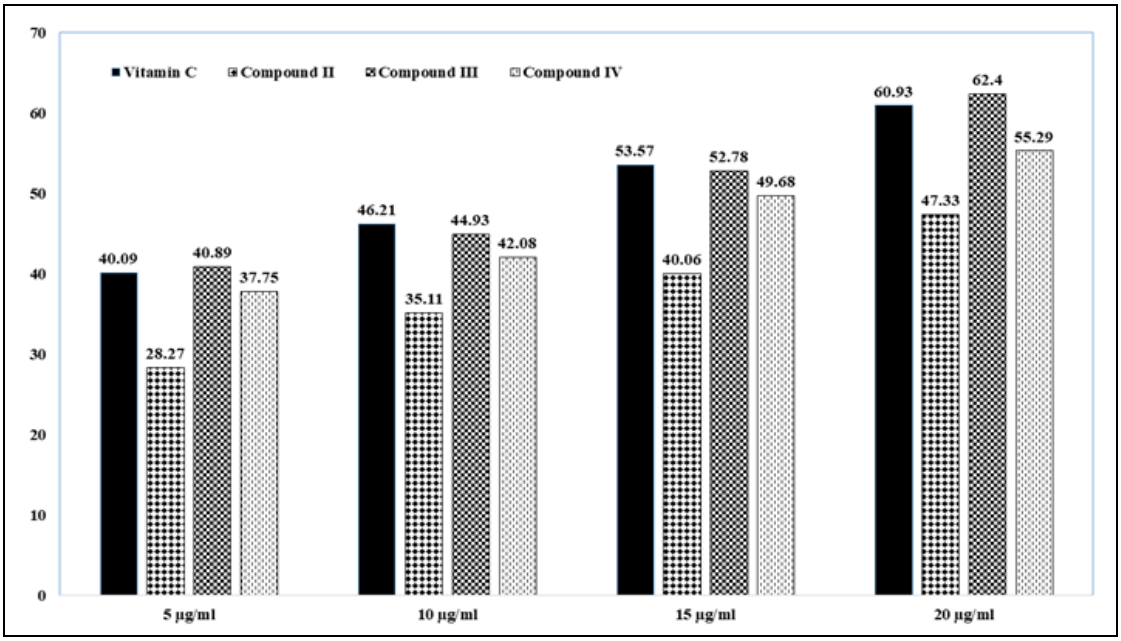


FIG. 2: NITRIC OXIDE RADICAL ACTIVITY

Hydroxyl Radical Scavenging Activity: The percentage inhibitions of all the compounds on hydroxyl radical was increased with increasing concentrations. The IC_{50} value for Hydroxyl radical scavenging activity of compound II was found to be 18.20, 26.49, 31.90, and 38.81 for 5, 10, 15, and 20 µg/ml concentrations, respectively. For compound III it was found to be 22. 23, 32.36, 36.71 and 43.90 for 5, 10, 15 and 20 µg/ml concentrations respectively while it was 17.42, 29.71, 32.63 and 37.67 for 5, 10, 15 and 20 µg/ml concentrations respectively for compound IV. The most effective and significant results were observed for compound III compared to a standard, i.e., 27.67, 34.50, 42.52, and 49.29 for 5, 10, 15, and 20 µg/ml concentrations **Fig. 3**.

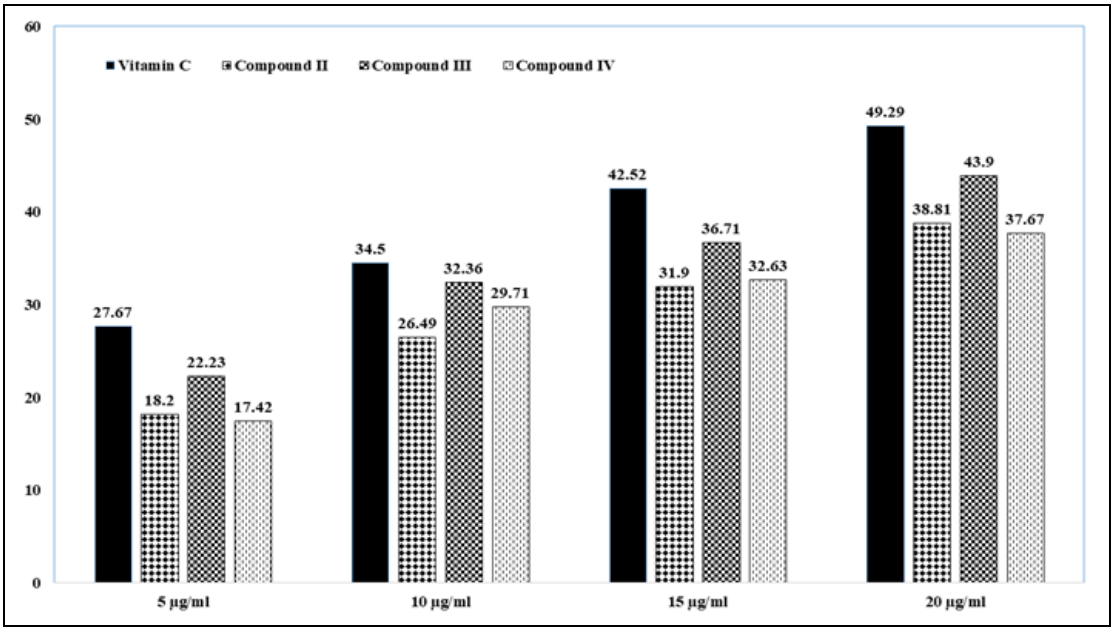


FIG. 3: HYDROXYL RADICAL SCAVENGING ACTIVITY

Hydrogen Peroxide Scavenging Activity: The scavenging ability against hydrogen peroxide scavenging assay is shown in **Fig. 4.** The highest inhibition for all the compounds was observed in 20µg/ml concentration, 53.09, 72.20, and 60.92 for compounds II, III, and IV, respectively. While, IC_{50} value for standard 73.88 at the same concentration **Fig. 4.** Antioxidants act as a defence mechanism that protects against deleterious effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system [19]. Oxidative stress is a primary cause of many disorders in humans, such as neurodegenerative diseases, cancer, and diabetes [20, 22]. Since scavenging of free radicals could inhibit the harmful effect of free radicals and stop the spreading of oxidation [23], antioxidants contents from plant origin through their scavenging activity are valuable for management of those diseases [22].

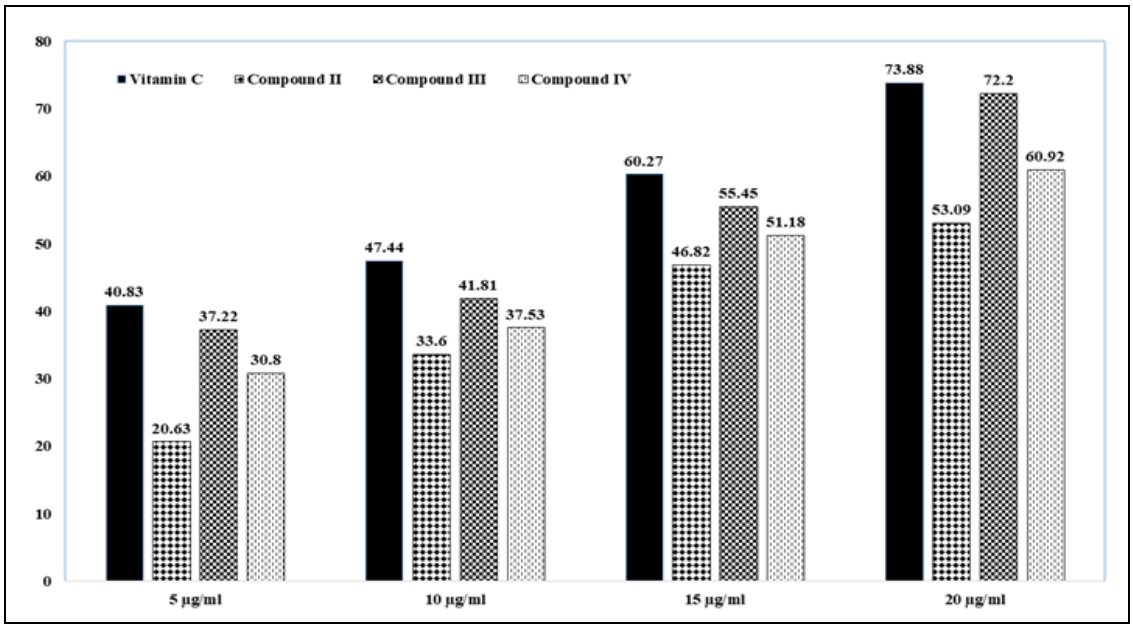


FIG. 4: HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY

CONCLUSION: A series of Lupeol derivatives were successfully synthesized in high to acceptable yields. The proposed structures of the Lupeol derivatives were confirmed by spectral analysis performed by IR, ^{1}H-NMR, 13C, Mass, and elemental analysis. It is thus concluded that lupeol skeleton deserves further investigation for the development of more potent and nontoxic new

agents for therapeutic use. Further optimization is needed to have a compound of a clinical trial. On the basis of the results obtained in the present study, it is concluded that compound III exhibits high scavenging activities compared to others and vitamin C, which was used as a reference standard. In conclusion, *in-vitro* assays indicate that these biologically active compounds could be a significant source of antioxidant, which might be helpful in preventing the progress of various oxidative stresses. However, other scavenging activities along with detail *in-vivo* studies are needed to develop into a drug.

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