Research Article

The Isolation, Characterization of Lupeol from *Crataeva nurvala* and Their Chemical Modification

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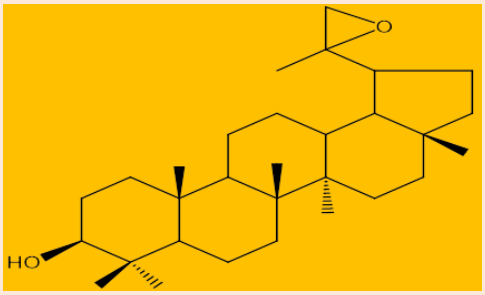
Abstract

Lupeol has been isolated from the bark of *Crataeva nurvala* and modified in various naturally occurring analogous derivatives. The structures of the newly obtained compounds were confirmed by elemental analysis, mass, IR and NMR spectra.



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Keywords: *Crataeva nurvala*, Lupeol, 3a, 5a, 5b, 8, 8, 11a-Hexamethyl-1-(2-methyl-oxiranyl)-icosa hydrocyclopenta[a] chrysen-9-ol, Lupeol acetate

Introduction

The plant *Crataeva nurvala* Buch Ham belongs to the family Capparidaceae. It is a leafy small to medium sized soft wooded tree with fragrant white flowers and oblong to rounded hard fruits, grows on the banks of canals, rivers, lakes etc [1]. The evergreen tree grows widely in all parts of Bangladesh, Pakistan, India, Philippine, South America, China, and Africa. It is commonly known as three leaved caper, holy garglic pear, lengam Tree, triune leaf tree, sacred lingam tree in English, barna, barun, bila, bilasi, biliana in Hindi, bhatavarna, hadavarna, kawan, kumla in Marathi, Maralingam in Tamil, barun tiktoshak in Bengali, varno, vayavarno in Gujarati, (Ghani, 2003). The bark of the tree is an important drug for problem affecting the kidneys and bladder. It is especially effective in the urinary complaints, kidney and bladder stones (inhibit the formation of stones), fever, vomiting and gastric irritation [2]. It also acts as contraceptive, juice of bark is given to women after childbirth. Root and bark are also laxative and lithontriptic. They increase appetite and billiary secretion [3]. Leaves are externally rubefacient and used in rheumatism; internally they are given as febrifuge and tonic.

Triterpenes are among the most abundant natural products in the plant kingdom and exhibit huge structural diversity [4]. Among them, pentacyclic triterpenes of the lupane type, such as lupeol, botulin and betulinic acid, have been reported to have interesting bioactivities including antiviral, in particular against to human immunodeficiency virus [5, 6], herpes simplex virus [7], and Epstein-Barr virus [8], anti-inflammatory [9] and antitumor against human melanoma and other types of human malignancies [10, 11]. Anti-inflammatory activity of lupeol and related compounds have been reported [12, 13]. Lupeol has been reported to show cardioprotective effects and was demonstrated to provide 34.4% protection against in vitro LDL oxidation [14]. Lupeol and lupeol acetate have also shown hypotensive activity, which may make them possible preventative agents in this cardiac disorder and other consequent cardiovascular diseases [15]. Lupeol and some analogues have demonstrated ability to function as antifertility agents. Lupeol acetate reduced male albino rats' fertility by 100%, which decreased testosterone levels and testicular weight in male rats [16, 17].

Thus it appear that this novel abundantly available 6-6-6-6-5-pentacyclic triterpene having a wide range of biological activities, can be used as template for a particular activity by grafting / crafting different pharmacophores, which can be optimized by chemical modification. i.e. one can generate pseudo hybrid natural products as novel bioactive compounds. In this paper we isolate lupeol from bark of *Crataeva nurvala* and synthesized three compounds from lupeol.

Materials and Methods

General experimental procedure

Column chromatography was performed on column (length 120 & diameter 2.5 cm), silica gel (60-120 mesh) and TLC on Merck's silica gel 60 F254 pre-coated glass plates. IR spectra were recorded on SHIMADZU FTIR-8400S spectrophotometer using KBr pellets. ^{1}H-NMR and ^{13}C- NMR spectra (300 MHz and 75 MHz respectively) were recorded on Brucker spectrometer using $CDCl_3$ as solvent and TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102/BA-600 mass spectrometer.

Extraction and isolation

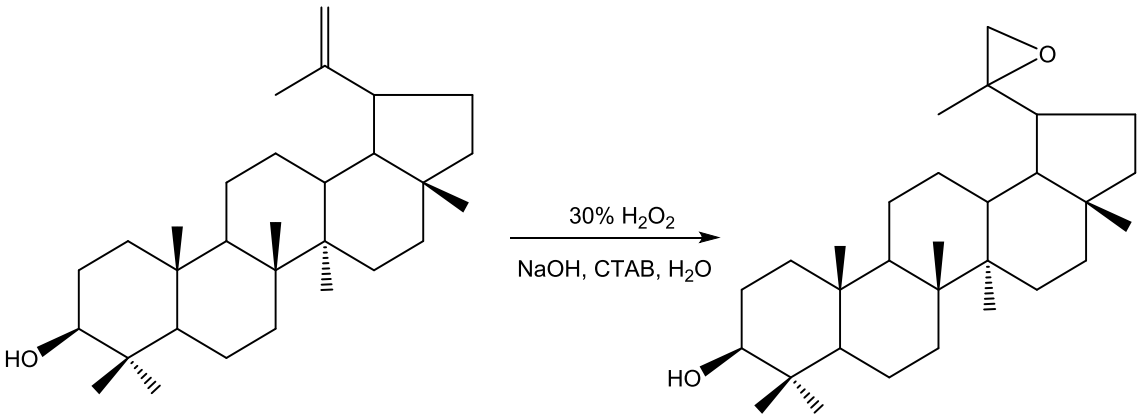
The Shade dried stem bark of *Crataeva nurvala* were powdered and extracted with petroleum ether on a water bath for 48 hours. The extract was concentrated under reduced pressure and chromatographed over silica gel (60-120 mesh) and eluted with the solvent, of increasing polarity. After elution lupeol was isolated and purified by PTLC followed by recrystallization. Lupeol was identified by ^{1}H NMR, ^{13}C NMR, and Mass spectral data.

Isolation of Lupeol (I)

It is isolated as colourless crystals, m.p. 220°C. It molecular formula is $C_{30}H_{50}O$. The spectral data are as: IR (KBr) 3450, 2940, 2845, 1640, 1380, 1355, 1315, 1280, 1275, 1110, 880. API-Mass (m/z) 426.0 (M^+), 408.4, 393.4, 327.2, 229.2, 218.1, 204.1, 189.1 (base peak), 175.1, 161.1, 147.1, 121.1, 107.0.

Synthesis of 3a, 5a, 5b, 8, 8, 11a-Hexamethyl-1-(2-methyl-oxiranyl)-icosahydrocyclopenta[a] chrysen-9-ol (II)

The lupeol with (1.0 mmol), aqueous 30% hydrogen peroxide (2.0 mmol), cetyletrimethylammonium bromide (0.05 mmol), water (5.0 mL), sodium hydroxide (1.0 mmol) were added into a 5 ml. round bottom flask and the reaction mixture was allowed to stir magnetically at 60°C for 6 hrs. 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).



I II

Scheme I Synthesis of 3a, 5a, 5b, 8, 8, 11a-Hexamethyl-1-(2-methyl-oxiranyl)-icosahydrocyclopenta[a] chrysen-9-ol

The characterization data of the compound are as given below: Light yellow crystals; m.p. 178°C; Yield: 63%

Analysis

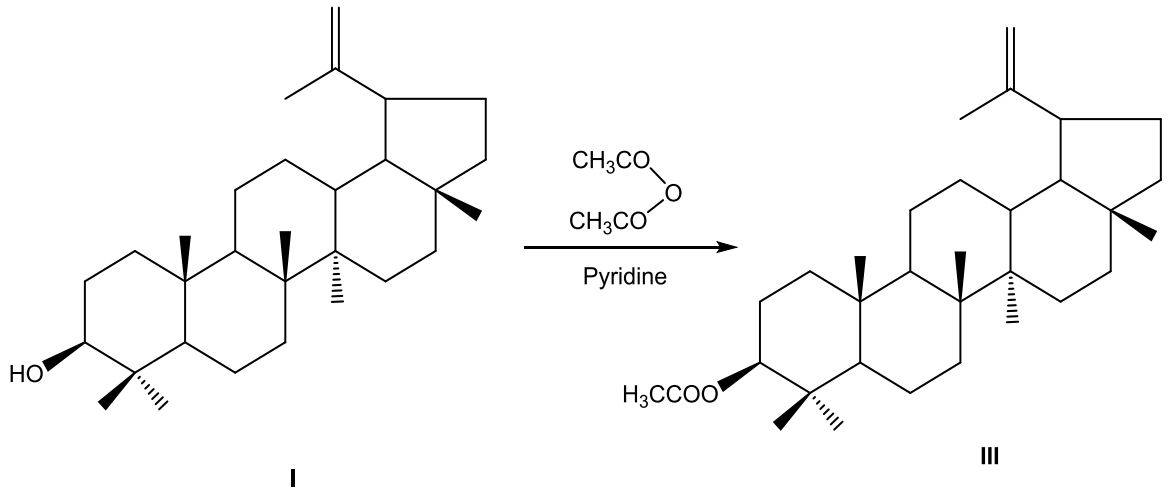
IR (KBr, cm^{-1}) vmax: 3290 (OH stretching), 1390 and 1365 (C-H stretching of >CMe_2 group), 1195 (–C–O–C– stretching), etc.

^{1}H NMR (δ, $CDCl_3$, 300.15MHz): 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-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).

^{13}C 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).

Synthesis of Lupeol acetate (III)

Lupeol (100 mg) and acyl chloride (300 mg) were taken in a round bottom flask. To this zinc dust (0.08g) was added and stirred for a 5 hr. The progress of the reaction was monitored by TLC. After completion of the reaction, ether was added and the organic layer was washed successively with saturated $NaHCO_3$ and water and dried over anhydrous Na_2SO_4. concentrated under vaccum and purified by silica gel column chromatography to afford pure lupeol acetate.



I III

Scheme II Synthesis of Lupeol acetate

The characterization data of the compound are as given below:

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 compound A was confirmed as $C_{32}H_{52}O_2$.

The IR spectrum (KBr, cm^{-1}) of the 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 (>CMe_2) in the title compound.

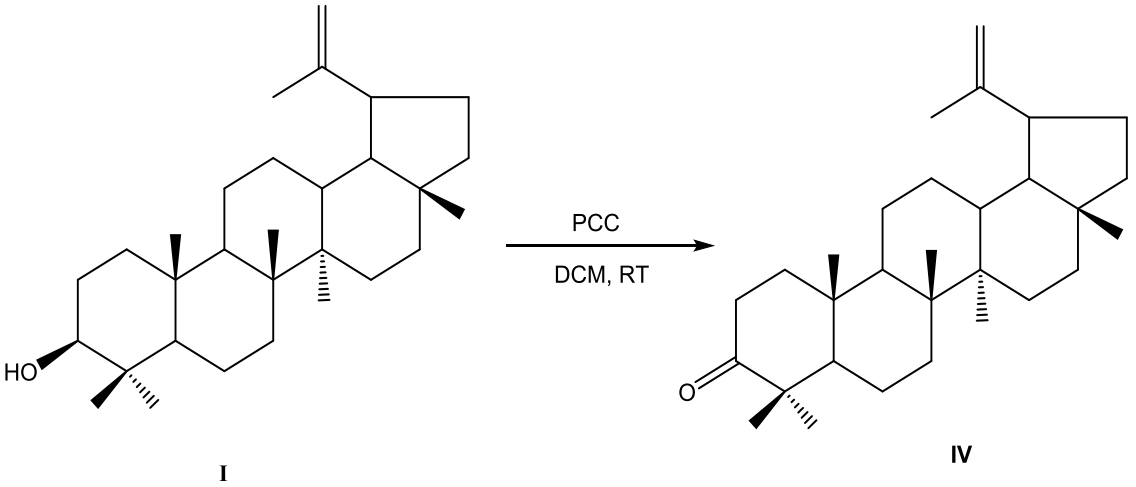
The proton NMR spectrum (δ ppm $CDCl_3$) showed sharp singlets at 0.78, 0.84, 0.87, 0.93, 0.96 and 1.04 indicated the presence of six methyl groups at C-24, C-27, C-25, C-26, C-28 and C-23 positions respectively. The three protons of methyl group at C-30 position was confirmed by the presence of a sharp singlet at 1.64. The presence of three protons of 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. The presence of two protons of pentacyclic ring at C-21 position was 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 remaining twenty-three protons was calculated in the region from 1.24 to 1.70.

In the ^{13}C NMR spectrum (δ ppm, $CDCl_3$), 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 olifinic carbon atom, appeared at 20.89. The presence of an 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

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).

Synthesis of Lupenone (IV)

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



Scheme III Synthesis of Lupenone

The characterization data of the compound are as given below:

In the mass spectrum, prominent molecular ion peak was appeared at m/z 422 [M^+]. The molecular formula of compound was assigned as $C_{30}H_{46}O$ by calculating protons and carbon atoms in the ^1H NMR and ^{13}C NMR spectrum respectively. The IR spectrum (cm^{-1}, KBr) showed characteristic absorption at 1745 suggested the presence of carbonyl group. The presence of >C=C< at C-20 (29) and C-12 (13) were confirmed by characteristic absorptions at 1610 and 1630 respectively. In the ^1H NMR spectrum (δ ppm, $CDCl_3$) three signals were observed at 0.80 (s, 3H), 0.87 (s, 6H) and 1.14 (s, 9H), were characterized for six tertiary methyl groups. Presence of a singlet at 1.61 confirmed the attachment of methyl groups to the olefinic carbon (C-20). A pair of broad singlet at 4.67 and 4.68 for one proton each was attributed for the vinylic protons. A multiplet between 2.21-2.52 was assigned for two protons at C-2 position. A singlet observed at 5.04 for one olefinic proton present at C-12 position. A doublet was observed at 1.71 for two protons at positions C-7. A complicated pattern was observed at 1.18-1.58 for eighteen protons. On the basis of above data and observation compound IV was characterized as lupa 12, 20 (29)-diene-3-one (m.p. 194°C). The spectral daa were compared with reported literature values.

Results and Discussion

Lupeol (1-isopropenyl)-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 is isolated as colourless crystals, m.p. 220°C. It molecular formula is $C_{30}H_{50}O$. 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 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 δ 0.75 (s, 3H, C-27), 0.77 (s, 3H, C-24), 0.84 (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. The 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 C-21 position in pentacyclic ring. The remaining twenty-three protons were observed in the region from 1.24 to 1.74. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) showed characteristic absorption for olefinic carbon atoms at 109.74 and 151.33. The attachment of hydroxyl group at C-3 position was confirmed by a single observed at 79.41.

These spectral data are in good agreement with those reported for lupeol in the literature [18]. On the basis of these observations compound was identified as lupeol.

The syntheses of natural lupeol analogues II-IV were accomplished by subjecting various chemical reactions. 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 been also modified in Lupenone by its oxidation through PCC. The isolated product (II to IV) were characterized on the basis of their spectral (IR, ^{1}H and ^{13}C NMR) studies.

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References

[1] Bhattacharjee A, Shashidhara SC, Aswathanarayana, Phytochemical and ethno-pharmacological profile of Crataeva nurvala Buch-Hum (Varuna): A review, Asian Pac J Trop Biomed 2012, 2(2), S1162-S1168.

[2] Khattar V, Wal A, Utilities of Crataeva nurvala, Int J Pharm Pharm Sci 2012, 4(4), 21-26.

[3] Saleem M, Lupeol, A Novel Anti-inflammatory and Anti-cancer Dietary Triterpene, Cancer Lett, 2009, 285(2), 109–115.

[4] Shibuya M, Xiang T, Katsube Y, Otsuka M, Zhang H, Ebizuka Y, Origin of Structural Diversity In Natural Triterpenes: Direct Synthesis of Seco-Triterpene Skeletons By Oxidosqualene Cyclase, J Am Chem Soc 2007, 129, 1450-1455.

[5] Barroso GJ, Jaber VNE, García EL, Machado JD, Zárate R, Ravelo AG, Estévez BA, Valenzuela FA, The Lupane-type Triterpene 30-oxo-calenduladiol is a CCR5 Antagonist with Anti-HIV-1 and Anti-chemotactic Activities, J Biol Chem 2009, 284, 16609-16620.

[6] Qian K, Yu D, Chen C, Huang L, Morris NSL, Nitz TJ, Salzwedel K, Reddick M, Allaway GP, Lee K, Anti-AIDS Agents. 78.† Design, Synthesis, Metabolic Stability Assessment, and Antiviral Evaluation of Novel Betulinic Acid Derivatives as Potent Anti-Human Immunodeficiency Virus (HIV) Agents, J Med Chem 2009, 52, 3248-3258.

[7] De Carvalho TC, Polizeli AM, Turatti ICC, Severiano ME, De Carvalho CE, Ambrósio SR, Crotti AEM, De Figueiredo US, Vieira PC, Furtado NAJC, Screening of Filamentous Fungi to Identify Biocatalysts for Lupeol Biotransformation, Molecules 2010, 15, 6140-6151.

[8] Nakagawa GK, Yamada K, Taniguchi M, Tokuda H, Lee K, Cancer Preventive Agents 9. Betulinic Acid Derivatives As Potent Cancer Chemopreventive Agents, Bioorg Med Chem Lett 2009, 19, 3378-3381.

[9] Nguemfo EL, Dimol T, Dongmo AB, Azebaze AGB, Alaoui K, Asongalem AE, Cherrah Y Kamtchouingl P, Anti-Oxidative and Anti-Inflammatory Activities of Some Isolated Constituents From The Stem Bark of Allanblackia monticola Staner L.C (Guttiferae), Inflammopharmacol 2009, 17, 37-41.

[10] Fulda S, Kroemer G, Targeting Mitochondrial Apoptosis By Betulinic Acid In Human Cancers, Drug Discov Today 2009, 14, 885-890.

[11] Saleem M, Murtaza I, Tarapore RS, Suh Y, Adhami VM, Johnson JJ, Siddiqui IA, Khan N, Asim M, Hafeez BB, Shekhani MT, Li B, Mukhtar H, Lupeol Inhibits Proliferation of Human Prostate Cancer Cells By Targeting B-Catenin Signalling, Carcinogenesis 2009, 30, 808-817.

[12] Bani S, Kaul A, Khan B, Ahmad SF, Suri KA, Gupta BD, Satti NK, Qazi GN, Suppression of T Lymphocyte Activity By Lupeol Isolated From Crataeva religiosa, Phytotherapy Res 2006, 20, 279-287.

[13] Vasconcelos JF, Teixeira MM, Barbosa FJM, Lúcio ASSC, Almeida JRGS, Queiroz LP, Ribeirodos SR, Soares MBP, The Triterpenoid Lupeol Attenuates Allergic Airway Inflammation In A Murine Model, Int Immunopharmacol 2008, 8, 1216-1221.

[14] Santiagoa LA, Mayora BAR, Lupeol: An antioxidant triterpene in Ficus pseudopalma Blanco (Moraceae), Asian Pacific Journal of Tropical Biomedicine, Volume 4, Issue 2, February 2014, Pages 109–118.

[15] Nayoung Han and Marica Bakovic, Biologically Active Triterpenoids and Their Cardioprotective and AntiInflammatory Effects, J Bioanal Biomed 2015, S12, 1-11.

[16] Gupta RS, Bhatnager AK, Joshi YC, Sharm MC, Khushalani V, Kachhawa JB, Induction of Antifertility with Lupeol Acetate in Male Albino Rats, Pharmacology 2005, 75, 57-62.

[17] Padashetty SA, Mishra SH, Effect of Terpenoidal Fraction of Echinops Echinatus Roots on Reproductive Parameters of Male Rats, J Nat Med 2007, 61, 452-457.
[18] Suleiman MM, Elgorashi EE, Samuel BB, Naidoo V, Eloff JN, Biological Activities of Compounds Isolated from Loxostylis alata (Anacardiaceae) Leaf Extract, Biochem Pharmacol 2013, 2(4), 1-6.

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