



A NEW PENTACYCLIC TRITERPENOID WITH ANTIMICROBIAL ACTIVITY FROM THE TUBERS OF *Cyperus rotundus* Linn.

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ABSTRACT

Keywords: *Cyperus rotundus* tubers, phytochemical, structure elucidation, lupenyl arabinosyl oleate

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Plan: The present study is aimed to carry out a phytochemical investigation on *Cyperus rotundus* Linn tubers and to conduct antimicrobial assays of the isolated phytoconstituents.

Methodology: The ethanolic extract of *Cyperus rotundus* tubers was prepared and used for the isolation of phytoconstituents. The structures of all the isolated compounds were elucidated on the basis of spectral data analysis such as IR, ¹H-NMR, ¹³C-NMR, Mass spectroscopy and chemical reactions. The isolated phytoconstituents were tested for antimicrobial activity against bacterial and fungal strains.

Outcome: Phytochemical investigation of *Cyperus rotundus* tubers resulted in isolation of four phytoconstituents characterized as *n*-trtriacontan-16-one (1), Lup-12, 20 (29)-dien-3β-ol-3-α-*L*-arabinofuranosyl-2'-octadec-9"-eionate (2), *n*-pentadecanyl-9-octadecenoate (3) and *n*-tetradecanyl-*n*-octadec-9, 12-dienoate (4). Only compound 2 showed antimicrobial activity. Lup-12, 20 (29)-dien-3β-ol-3-α-*L*-arabinofuranosyl-2'-octadec-9"-eionate is a new phytoconstituent isolated for the first time from the plant source. Compound 1, 3 and 4 are reported first time from the *Cyperus* genus. Phytochemical investigation of tubers of *Cyperus rotundus* led to isolation of a new triterpenic constituent which may be used as chromatographic markers for quality control of the drugs.

1. INTRODUCTION

Cyperus rotundus Linn. (Family Cyperaceae), commonly known as 'Mustaka' is a pestiferous perennial weed with dark green glabrous culms, arising from underground tubers found throughout India. The tubers are credited with diuretic, anthelmintic, carminative, stomachic, emmenagogue, stimulant, anti-inflammatory and hypotensive properties.¹⁻²

Traditionally, it has been also used for the treatment of diabetes. Oral daily administration of *C. rotundus* extract significantly lowered the blood glucose level in alloxan-induced hyperglycemia in rats.³ Other pharmacological investigations indicated that *C. rotundus* had remarkable antipyretic⁴, antidiarrhoeal⁵, antimalarial⁶ and wound healing activity.⁷ Earlier investigation on this plant led to isolation of α -rotunol, β -rotunol⁸, cyperolone⁹, 2 α -(5-oxypentyl)-2 β -methyl-5 β -isopropenylcyclohexanone¹⁰, rotundene, rotundenol¹¹, 4 α ,5 α -oxidoeudesm-11-en-3 α -ol¹², α -cyperone⁶, patchoulone, caryophyllene- α -oxide, 10,12-peroxycalamenene, 4,7-dimethyl-1-tetralone¹³, norcyperone¹⁴, Oleanolic acid-3-O-neohesperidoside¹⁵ and sitosteryl (6'-hentriacontanoyl)-beta-D-galactopyranoside.¹⁶

The present paper describes the isolation and characterization of a unknown lupeol type pentacyclic triterpenoid Lup-12, 20 (29)-dien-3 β -ol-3- α -L-arabinofuranosyl-2'-octadec-9"-eionate,(2) from the tubers of *C. rotundus* along with the three known compounds n-tritriacontan-16-one, n-pentadecanyl-9-octadecenoate and n-tetradecanyl-n-octadec-9,12-dienoate.

2. MATERIALS AND METHODS

2.1. General experimental procedures

Melting points were determined on a Perfit apparatus without correction. The IR spectra were measured in KBr pellet on a Perkin Elmer-Spectrum RX-IFTIR spectrometer. Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer. ¹H (400 MHz), ¹³C (100 MHz) spectra were recorded on Bruker spectropin NMR spectrometer model Avance II. CDCl₃ (Sigma-Aldrich, Bangalore, India) was used as solvent and TMS as an internal standard. FAB MS analyses were performed on a JEOL SX 102/Da-600 instrument equipped with direct inlet probe system. Column chromatography separations were carried out on silica gel (Merck, 60–120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60 F254) were used for analytical thin layer chromatography visualized by exposure to iodine and UV radiations.

2.2. Plant material

The plant material was collected at its flowering stage from Hisar district, Haryana, July 2009 (29°10'12" N Latitude and 75°43'12" Longitude E). The plant was identified and authenticated by the Dr. H. B. Singh, Head, Raw Materials Herbarium & Museum, NISCAIR, New Delhi, Ref. No. NISCAIR/RHMD/Consult/2009-10/1491/89. A voucher specimen has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Guru Jambheshwar University of Science & Technology, Hisar, Haryana.

2.3. Preparation of extract

The air-dried tubers (3.5 kg) of *Cyperus rotundus* Linn were coarsely powdered and extracted exhaustively with ethanol using Soxhlet apparatus for 72 h. The extract was concentrated under reduced pressure to get dark brown mass (280 g, yield 8%).

2.4. Isolation

Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The extract was dissolved in minimum amount of methanol and adsorbed on silica gel (60–120 mesh) for preparation of slurry. The slurry was dried and subjected to silica gel column chromatography. The development and the elution of the column were carried with successive series of solvents in increment of polarity *viz.* petroleum ether, petroleum ether: chloroform (99:1, 98:2, 95:5, 90:10, 85:15, 75:25, 50:50, 25:75, 85:15), chloroform, chloroform: methanol (99:1, 98:2, 95:5, 90:10, 85:15, 75:25, 50:50, 25:75, 85:15) and methanol. The fractions collected were subjected to thin layer chromatography to check the homogeneity of various fractions. The fractions having the same R_f values were combined together. Purification of the isolated compounds was achieved by crystallization to obtain the following compounds.

2.5. *n*-tritriacontan-16-one (compound 1)

Elution of the column with petroleum ether furnished colorless powder of compound *n*-tritriacontan-16-one recrystallized from acetone: methanol (1:1), 248 mg (0.0083%), R_f 0.38 (petroleum ether), m.p. 55–58°C, UV λ_{max} (MeOH): 221 nm. IR ν_{max} (KBr): 2923, 2853, 1710, 1462, 1377, 1243, 1174, 1055, 721 cm^{-1} . 1H -NMR ($CDCl_3$): δ 2.32 (2 H, m, H_2 -15), 2.14 (2 H, m, H_2 -17), 1.70 (2 H, m, CH_2), 1.63 (2 H, m, CH_2), 1.35 (2 H, m, CH_2), 1.27 (6 H, br s, 3 x CH_2), 1.21 (44 H, br s, 22 x CH_2), 0.88 (3 H, t, $J = 6.3$ Hz, Me-33), 0.84 (3 H, t, $J = 6.1$ Hz, Me-1). ^{13}C NMR ($CDCl_3$): δ 191.75 (C-16), 31.95 (CH_2), 29.72 (27 x CH_2), 29.39 (CH_2), 22.72 (CH_2), 14.91 (Me-1), 14.16 (Me-33). +ve FAB-MS m/z (rel. int.): 478 $[M]^+$ ($C_{33}H_{66}O$) (1.2), 239 (11.3).

2.6. Lupenyl arabinosyl oleate (compound 2)

Elution of the column with petroleum ether: chloroform (7:3) furnished colorless crystals of compound lupenyl arabinosyl oleate recrystallized from methanol, 232 mg (0.0077%), R_f 0.36 (petroleum ether: chloroform 7:3), m.p. 232–234°C, UV λ_{max} (MeOH): 236 nm. IR ν_{max} (KBr): 3465, 3325, 2918, 2849, 1736, 1640, 1462, 1376, 1180, 720 cm^{-1} . 1H -NMR ($CDCl_3$): δ 5.36 (1H, d, $J = 5.0$ Hz, H-12), 5.34 (1H, m, H-9"), 5.32 (1H, m, H-10"), 5.13 (1H, d, $J = 7.2$ Hz, H-1'), 4.72 (1H, br s, H_2 -29a), 4.67 (1H, br s, H_2 -29b), 4.58 (1H, dd, $J = 7.2, 4.5$ Hz, H-2'), 4.54 (1H, m, H-4'), 4.13 (2H, t, $J = 7.0$ Hz, H_2 -5'), 4.06 (1H, dd, $J = 5.0, 7.1$ Hz, H-3 α), 3.40 (1H, m, H-3'), 2.30 (2H, t, $J = 7.2$ Hz, H_2 -2"), 2.27 (2H, m, H_2 -8), 2.17 (2H, m, H_2 -11), 1.62 (3H, br s, Me-30), 1.02 (3H, br s, Me-23), 0.97 (3H, br s, Me-25), 0.89 (3H, br s, Me-27), 0.87 (3H, br s, Me-28), 0.85 (3H, br s, Me-26), 0.83 (3H, br s, Me-24), 0.80 (3H, t, $J = 6.1$ Hz, Me-18"). ^{13}C -NMR ($CDCl_3$) spectral data Table 1. +ve FAB-MS m/z (rel. int.): 821 $[M+H]^+$ ($C_{53}H_{89}O_6$) (2.1), 451 (20.5), 282 (34.6)

2.7. Pentadecanyl-9-octadecenoate (compound 3)

Elution of the column with chloroform: methanol gave light yellow amorphous powder of compound Pentadecanyl oleate recrystallized from methanol, 150 mg (0.005%), R_f 0.72 (chloroform: methanol 9:1), m.p. 175–178°C, UV λ_{max} (MeOH): 217 nm.

IR ν_{\max} (KBr): 2920, 2850, 1736, 1653, 1462, 1375, 1246, 1174, 1114, 889, 729 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3): δ 5.37 (1H, m, H-9), 5.34 (1H, m, H-10), 4.14 (2H, t, $J = 7.2$ Hz, H_2 -1'a), 4.11 (2H, t, $J = 7.2$ Hz, H_2 -1'b), 2.32 (2H, t, $J = 7.2$ Hz, H_2 -2'a), 2.29 (2H, t, $J = 7.2$ Hz, H_2 -2'b), 2.18 (2H, m, H_2 -8), 2.02 (2H, m, H_2 -11), 1.62 (4H, m, 2 x CH_2), 1.31 (8H, br s, 4 x CH_2), 1.29 (10H, br s 5 x CH_2), 1.27 (20H, br s 10 x CH_2), 1.22 (6H, br s 3 x CH_2), 0.88 (3H, t, $J = 6.5$ Hz, Me-18), 0.84 (3H, t, $J = 6.2$ Hz, Me-15'). $^{13}\text{C-NMR}$ (CDCl_3): δ 171.27 (C-1), 126.13 (C-9), 125.57 (C-10), 63.13 (C-1'), 32.85 (CH_2), 31.95 (CH_2), 29.72 (23 x CH_2), 29.39 (CH_2), 22.71 (CH_2), 14.31 (Me-18), 14.15 (Me-15'). +ve FAB-MS m/z (rel. int.): 492 [M]⁺ ($\text{C}_{33}\text{H}_{64}\text{O}_2$) (2.1).

2.8. Teratadecanyl linoleate (compound 4)

Elution of the column with chloroform: methanol furnished colorless crystal of tetradacanyl linoleate recrystallized from acetone: methanol (1:1), 182 mg (0.0061%), R_f 0.42 (chloroform: methanol 9:1), m.p. 202-205°C, UV λ_{\max} (MeOH): 234 nm. IR ν_{\max} (KBr): 2918, 2849, 1737, 1625, 1463, 1377, 1244, 1172, 889, 729 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3): δ 5.39 (1 H, m, H-9), 5.36 (2 H, m, H-10, H-12), 5.34 (1 H, m, H-13), 4.15 (1 H, d, $J = 7.2$ Hz, H_2 -1'a), 4.09 (1 H, d, $J = 6.8$ Hz, H_2 -1'b), 2.33 (2 H, k, $J = 7.1$ Hz, H_2 -2), 2.08 (2 H, m, H_2 -11), 1.67 (4 H, br s, H_2 -8, H_2 -14), 1.60 (6 H, br s, 3 x CH_2), 1.37 (6 H, br s, 3 x CH_2), 1.27 (28 H, br s, 14 x CH_2), 0.89 (3 H, t, $J = 6.5$ Hz, Me-18), 0.86 (3 H, t, $J = 6.3$ Hz, Me-14'). ^{13}C NMR (CDCl_3): δ 173.05 (C-1), 133.26 (C-9), 128.21 (C-10), 125.89 (C-12), 118.03 (C-13), 64.52 (C-1'), 33.48 (CH_2), 32.19 (CH_2), 31.95 (CH_2), 29.72 (20 x CH_2), 29.69 (CH_2), 29.39 (CH_2), 22.72 (CH_2), 14.35 (Me-18), 14.15 (Me-14'). +ve FAB-MS m/z (rel. int.): 476 [M]⁺ ($\text{C}_{32}\text{H}_{60}\text{O}_2$) (14.8), 279 (8.9), 263 (5.8).

2.9. Antimicrobial assays

The following bacterial and fungal strains were used in the study, *Staphylococcus epidermidis* MTCC-3615, *Bacillus cereus* MTCC-430, *Pseudomonas aeruginosa* MTCC-424, *Escherichia coli* MTCC-443, *Aspergillus niger* MTCC-1344 and *Candida albicans* MTCC-227. The antibacterial activity of all compound were studied by agar well diffusion method²² with slight modification. Molten nutrient agar (25 ml) was poured into presterilized petriplates and allowed to solidify at room temperature. Potato dextrose agar was used for fungal cultures. The plates were then seeded with 0.1ml (10^5 - 10^6 cells/ml) of overnight bacterial culture. Subsequently 8 mm wide wells were bored within these agar plates using a sterile cork borer. The wells were aseptically filled with 100 μ l of various concentrations of compound **2** and labeled accordingly. The plates were incubated over night at 37°C for bacterial cultures and at 20-22°C for 5 days for fungal cultures. Microbial growth was determined by measuring the diameter of zone of inhibition. For each strain, a negative control was maintained where DMSO without compound was used. Standard antibiotic like gentamycin and amphotericin were also maintained as positive control (Figure 2). The above experiment was carried out three times and mean values are presented herewith.

3. RESULTS AND DISCUSSION

Compound n-tritriacontan-16-one, (**1**) was obtained from petroleum ether eluent. Its IR spectrum showed distinct absorption bands for keto group (1710 cm^{-1}) and long aliphatic chain (721 cm^{-1}). It has a molecular ion peak in the mass spectrum at m/z 478 corresponding to aliphatic ketone, $\text{C}_{33}\text{H}_{66}\text{O}$.

The ion peak generating at m/z 239 $[\text{CH}_3(\text{CH}_2)_{14}\text{CO}]^+$ indicated that the keto function was present at C-16. The ^1H NMR spectrum of C-1 exhibited methylene protons from δ 2.32 to 1.21 and primary methyl proton as three-proton triplets at δ 0.88 ($J = 6.3\text{ Hz}$, Me-33) and 0.84 ($J = 6.1\text{ Hz}$, Me-1). The ^{13}C NMR spectrum of C-1 displayed signals for keto carbon at δ 191.75 (C = 16), methylene carbon between δ 31.95-22.72 and methyl carbons at δ 14.91 (C-1) and 14.16 (C-33).

Compound lupenyl arabinosyl oleate, (**2**) was obtained as a colorless crystalline mass from petroleum ether: chloroform (7:3) eluents. It gave positive test for triterpenic glycoside¹⁸. Its IR spectrum showed characteristic absorption bands for hydroxyl groups ($3465, 3325\text{ cm}^{-1}$), ester function (1736 cm^{-1}), unsaturation (1640 cm^{-1}) and long aliphatic chain (720 cm^{-1}). The mass spectrum displayed a molecular ion peak at m/z 821 $[\text{M}+\text{H}]^+$. On the basis of MS and ^{13}C NMR spectral data, it corresponds to the molecular formula of a triterpenic glycosidic ester, $\text{C}_{53}\text{H}_{89}\text{O}_6$. The ion peak arising at m/z 282 $[\text{CH}_3\text{C}_{16}\text{H}_{30}\text{COOH}]^+$ and 451 $[\text{M}-\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOC}_5\text{H}_8\text{O}_3]^+$ suggested that oleic acid was esterified with the sugar unit which was linked to the lupine-type triterpene¹⁹. The ^1H NMR spectrum showed two one proton doublets at δ 5.36 ($J = 5.0\text{ Hz}$) and 5.13 ($J = 7.2\text{ Hz}$) and two one proton multiplets at δ 5.34 and 5.32 assigned to vinylic H-12, anomeric H-1' and vinylic H-9" and H-10" protons respectively. Two one-proton broad signals at δ 4.72 and 4.67 were due to vinylic H₂-29 methylene protons indicating lupene-type of triterpenic unit. A one proton double doublet at δ 4.58 ($J = 7.2$ and 4.5 Hz) was ascribed to sugar H-2' carbinol proton and its deshielding nature suggested the location of ester formation at C-2'. The other sugar proton appeared as one-proton multiplets at δ 4.54 (H-4') and 3.40 (H-4') as a two proton triplet at δ 4.13 ($J = 7.0\text{ Hz}$, H-5'). A one proton double doublet at δ 4.06 with coupling interactions of 5.0, 7.1 Hz was accounted to oxygenated C-3 α methine proton. A two-proton triplet at δ 2.30 ($J = 7.2\text{ Hz}$) was due to methylene H₂-2" protons adjacent to the ester group. A three proton singlet in the deshielded region at δ 1.62 was attributed to C-30 methyl protons located on a vinylic carbon. The other methyl signals appeared as three proton broad singlets between δ 1.02-0.83. A three proton triplet at δ 0.80 ($J = 6.1\text{ Hz}$) was assigned to C-18" primary methyl protons. The ^{13}C NMR spectrum (Table 1) of this compound showed signals for ester carbon at δ 173.99 (C-1"); vinylic carbons at δ 122.60 (C-12), 139.68 (C-13), 156.93 (C-20) and 108.21 (C-29); anomeric carbon at δ 105.93 (C-1'), oxygenated triterpenic methine carbon at δ 73.60 (C-3), and other sugar carbons at δ 80.33 (C-2'), 64.43 (C-3'), 87.21 (C-4'), 60.18 (C-5'). The shifting of ^1H NMR signal at δ 4.58 (H-2') and ^{13}C NMR signal at δ 80.33 (C-2') in the deshielded region suggested the location of the acyl group at C-2'. The presence of the sugar carbons C-2' and C-4' in the deshielded region supported furanic form of the sugar unit. The carbon signals of the triterpenic unit were compared with related lupene-type molecules²⁰⁻²². Alkaline hydrolysis of this compound yielded Lup-12, 20 (29)-dien-3 β -ol, α -L arabinose and oleic acid.

On the basis of spectral data analysis and chemical reactions, the structure had been formulated as Lup-12, 20 (29)-dien-3 β -ol-3- α -L-arabinofuranosyl-2'-octadec-9"-eionate. This is a new lupine type triterpenic glycoside isolated first time from plant source.

N-pentadecanyl-9-octadecenoate, (**3**) was obtained as a colorless crystalline mass from chloroform: methanol (99:1) elute. It decolourized bromine water indicating the unsaturated nature of the molecule. Its IR spectrum showed characteristic absorption bands for ester group (1736 cm⁻¹) and unsaturation (1653 cm⁻¹). The mass spectrum displayed a molecular ion peak at *m/z* 492 corresponding to molecular formula of unsaturated fatty acid ester, C₃₃H₆₄O₂. It had two double bond equivalents which were adjusted in the vinylic linkage and ester group. The ¹H-NMR spectrum of this compound exhibited two one-proton multiplets at δ 5.37 and 5.34 assigned to vinylic H-9 and H-10 protons.

Four one proton doublets at δ 4.14 (*J* = 7.2 Hz) and 4.11 (*J* = 7.2 Hz) and at 2.33 (*J* = 7.2 Hz) and 2.29 (*J* = 7.2 Hz) were attributed correspondingly to oxygenated methylene protons H₂-1' and methylene protons H₂-2 protons adjacent to the ester group. Two multiplets at δ 2.18 and 2.02, both integrated for two protons each, were accounted to methylene protons adjacent to the vinylic carbons. Two three-proton triplets at δ 0.88 (*J* = 6.5 Hz) and 0.85 (*J* = 6.2 Hz) were associated to primary C-18 and C-15 methyl protons, respectively, C-18 and C-15.

The remaining methylene protons resonated between δ 1.62-1.22. Its ¹³C-NMR spectrum exhibited important signals for ester carbon at δ 171.27 (C-1), vinylic carbons at δ 126.13 (C-9) and 125.57 (C-10), oxygenated methylene carbons at δ 63.13 (C-1'), methyl carbons at δ 14.31 (C-18) and 14.15 (C-15') and the remaining methylene carbons between δ 32.85-22.71. Acid hydrolysis of n-pentadecanyl-9-octadecenoate yielded oleic acid (TLC comparable).

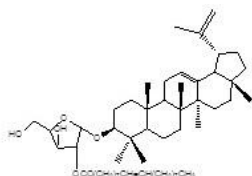
Compound tetradacanyl linoleate, (**4**) was obtained from chloroform: methanol (49:1) eluent. Its IR spectrum showed characteristic absorption bands for ester group (1737 cm⁻¹), unsaturation (1625 cm⁻¹) and long aliphatic chain (729 cm⁻¹). It had molecular ion peak in the mass spectrum at *m/z* 476 corresponding to fatty ester, C₃₂H₆₀O₂. The ion peaks arising at *m/z* 263 [CH₃ (C₁₆H₂₈) CO]⁺ and 279 [CH₃ (C₁₆H₂₈) COO]⁺ indicated that linoleic acid was esterified with C₁₄ alcohol. The ¹H NMR spectrum showed vinylic protons as multiplets at δ 5.39 (H-9), 5.36 (H-10, H-12) and 5.34 (H-13), oxygenated methylene protons as doublets at δ 4.15 (*J* = 7.2 Hz) and 4.09 (*J* = 6.8 Hz) ascribed to oxygenated methylene H₂-1' proton, other methylene protons between δ 2.33-1.27 and primary methyl protons as three-proton triplets at δ 0.89 (*J* = 6.5 Hz, Me-18) and 0.86 (*J* = 6.3 Hz, Me-14'). The ¹³C NMR spectrum exhibited signals for ester carbon at δ 173.05 (C-1), vinylic carbons between 133.26-118.03, oxygenated methylene at δ 64.52 (C-1'), other methylene carbons from δ 33.48 to 22.72 and methyl carbons at δ 14.35 (C-18) and 14.15 (C-14'). On the basis of above discussion, the structure had been formulated as n-tetradecanyl-n-octadec-9, 12-dienoate²³.

All the isolated compounds were evaluated for antimicrobial activity against gram (+ve), gram (-ve) bacteria and fungi. Only compound 2 was found be active.

The result reported in Figure 2 showed inhibition of growth for some of the tested microorganisms with various degrees. Compound 2 was found to be highly active against gram (+ve) *Staphylococcus epidermidis* for antibacterial activity and *Aspergillus niger* for antifungal activity.

3.1. Compounds isolated from *Cyperus rotundus* tubers

Compound 1. N-tritriacontan-16-one $\text{CH}_3(\text{CH}_2)_{14}\text{CO}(\text{CH}_2)_{16}\text{CH}_3$



Compound 2. Lupenyl arabinosyl oleate:

Compound 3. N-pentadecanyl-9-octadecenoate: $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOCH}_2(\text{CH}_2)_{13}\text{CH}_3$

Compound 4. Tetradacanyl linoleate: $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOCH}_2(\text{CH}_2)_{12}\text{CH}_3$

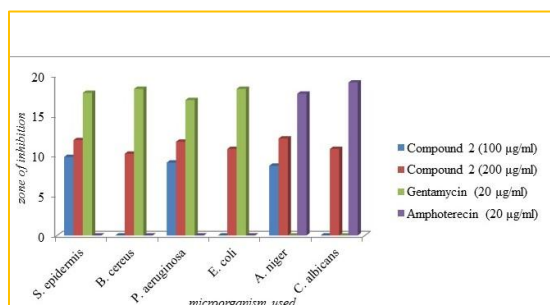


Figure 2- Antimicrobial activity of compound 2

Table 1. ^{13}C NMR spectral data of lupenyl arabinosyl oleate

^{13}C NMR CDCl_3					
Position	δ_c	Position	δ_c	Position	δ_c
1	36.13	19	45.29	2"	34.89
2	26.49	20	156.93	3"	29.82
3	73.60	21	31.32	4"	29.73
4	36.66	22	42.17	5"	29.68
5	48.81	23	29.13	6"	29.62
6	18.01	24	15.23	7"	29.55
7	31.95	25	18.31	8"	34.42
8	39.48	26	20.94	9"	130.01
9	47.90	27	25.17	10"	129.78
10	37.01	28	21.03	11"	32.85
11	22.72	29	108.21	12"	29.39
12	122.60	30	19.31	13"	29.29
13	139.68	1'	105.93	14"	29.17
14	45.29	2'	80.33	15"	28.66
15	28.66	3'	64.43	16"	29.82
16	34.96	4'	87.21	17"	20.12
17	56.68	5'	60.18	18"	14.16
18	52.24	1"	173.99		

4. CONCLUSION

The present work characterized four phytoconstituents from the tubers of *C. rotundus*. Lup-12, 20 (29)-dien-3 β -ol-3- α -L-arabinofuranosyl-2'-octadec-9"-eionate is a new phytoconstituent isolated for the first time from the plant source. N-tritriacontan-16-one, n-pentadecanyl-9-octadecenoate and n-tetradecanyl-n-octadec-9, 12-dienoate are reported first time from the *Cyperus* genus. The existing knowledge about the phytoconstituents of *Cyperus* genus is increased by phytochemical investigation of *C. rotundus*. These phytoconstituents may be used as finger printing markers for quality control of *C. rotundus*. From the studies of antimicrobial assays, it can be conclude that Lup-12, 20 (29)-dien-3 β -ol-3- α -L-arabinofuranosyl-2'-octadec-9"-eionate may potentially be used as active agent against diseases caused by these microorganisms.

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