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NEW ANTIFUNGAL TRITERPENOID SAPONIN FROM LEAVES OF *BLUMEA ERIANTHA* DC

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ABSTRACT

A new triterpenoid saponin 3β, 28-dihydroxy-20(29)-lupene-3-O-β-D-galactopyranosyl-(1→4)-O-β-D-xylopyranosyl-28-O-α-L-rhamnopyranoside (**1**) together with known compounds ursolic acid, α-amyrine acetate and lupeol were isolated from ethanolic extract of the leaves of *Blumea eriantha* DC. Their structures were elucidated by various colour reactions, spectral analysis and chemical degradations. Compound **1** showed antifungal activity.

KEYWORDS: *Blumea eriantha* DC, compositae, triterpenoid saponin, antifungal activity.

INTRODUCTION

Blumea eriantha DC[1-2] belongs to family compositae, which is commonly known as "**Janglimuli**" or "**Kakronda**" in Hindi, "**Jangali Tambaku**" in Kannada and "**Nimardi**" in Marathi. It is a small annual herb up to 1 m in height, often dichotomously branched, covered with white and silky hairs, possessing camphor like smell. It is commonly found in tropical countries. It is widespread in India especially Maharashtra, Karnataka, Madhya Pradesh, dry region of Uttar Pradesh and Orissa. Juice of this herb is used as a carminative. A warm infusion is given as a sudorific in catarrhal affections. The essential oil extracted from the leaves and stem show potent antibacterial, antifungal and insecticidal properties. This plant has been traditionally used as a diuretic and also for the treatment of cholera and diarrhea.[3-4] Earlier workers[5-9] have reported various chemical constituents from this plant. In the present paper we report the isolation and structural elucidation of a new triterpenoid saponin 3β, 28-dihydroxy-20(29)-lupene-3-O-β-D-galactopyranosyl-(1→4)-O-β-

D-xylopyranosyl-28-O-α-L-rhamnopyranoside (**1**) alongwith known compounds ursolic acid (**2**) α-amyrine acetate (**3**) and lupeol (**4**) from ethanolic extract of the leaves of this plant.

RESULTS AND DISCUSSION

Chemical examination of ethanolic extract of leaves of *Blumea eriantha* DC yielded a new compound **1**. It has molecular formula $C_{47}H_{78}O_{15}$, m. p. 224-226^{O}C, $[M]^+$ 882(FABMS). Positive results with Liebermann-Burchard[10], foam test[11] and molisch's reagents evidented its triterpenoidal saponin glycosidic nature. It also gave positive molisch test. The IR spectrum of compound **1** revealed an absorption band at 3354 cm^{-1}(OH), 1688 (double bond), 1368 (gem dimethyl) and 1029 (suggestive of a glycosidic compound). An intense band at 882 cm^{-1} is caused by the CH_2 vibration of the alkene group. In ^{1}H-NMR spectrum a double doublet at δ3.16 (1H, dd, *J* 9.8, 5.2 Hz) was assigned to proton at C-3 position. Two downfield olefinic methylene proton signals at δ 4.68 and 4.55 assigned an isopropenyl unit for H-29$_a$ and H-29$_b$. Six singlets at δ 0.90, 0.71, 0.74, 0.93, 0.95 and 1.64 showed the presence of six methyl groups at C-23, C-24, C-25, C-26, C-27 and C-30 of triterpenoid skeleton. The anomeric proton signals at δ 4.78(1H, d, *J* 7.4 Hz, H-1′), 5.10(1H, d, *J* 7.5 Hz, H-1″) and 5.03(1H, d, *J* 1.6 Hz, H-1‴) were assigned for H-1', H-1" and H-1'" of D-xylose, D-galactose and L-rhamnose respectively. In ^{13}C-NMR spectrum signals at δ 150.4 and 109.5 assigned carbon-carbon double bond between C-20 and C-29. The downfield ^{13}C-NMR chemical shift at δ 79.3 and δ 60.4 suggested that compound **1** was a triterpenoid saponin with glycosidic linkage at C-3 and C-28 through an ether bond.

In the mass spectrum of the compound **1**, characteristic ion peaks at [M^+] m/z 882, 736[M^+-L-rhamnose], 574[M^+-D-galactose] and 442[M^+-D-xylose, aglycone] were found by subsequent losses from the molecular ion of each molecule of L-rhamnose, D-galactose and D-xylose revealing L-rhamnose was attached at C-28 position, D-galactose was terminal sugar at C-3 position and D-xylose was attached at C-3 position of aglycone.

Acid hydrolysis of compound **1** with 10% ethanolic H_2SO_4 gave aglycone **1-A**, m.p. 247-248°C , m.f. $C_{30}H_{50}O_2$, $[M]^+$442 (EIMS). It responded all the colour rections of triterpenoids.[12] It was identified as 3β, 28-dihydroxy-20(29)-lupene by comparision of its spectral data with reported literature values.[13-16]

The aqueous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination and sugars were identified as D-galactose (R_f 0.16), D-xylose (R_f0.28) and L-rhamnose (R_f 0.36) (Co-PC and Co-TLC).[17] Periodate oxidation of compound **1** confirmed that all the sugars were present in the pyranose form.[18]

Permethylation[19] followed by acid hydrolysis of compound **1** yielded aglycone identified as 3β, 28-dihydroxy-20(29)-lupene showed that glycosylation was involved at C-3 and C-28. Methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.02), 2, 3, 4, 6-tetra-O-methyl-D-galactose (R_G 0.88) and 2, 3-di-O-methyl- D-xylose (R_G 0.72), indicating that C-1'"-OH of L-rhamnose was linked with C-28 position of the aglycone. C-1"-OH of D-galactose was linked to C-4'-OH of D-xylose and C-1'-OH of D-xylose was linked with C-3 position of the aglycone. Therefore it was concluded that interlinkage (1→4) between D-galactose and D-xylose. The linkages were further confirmed by spectral data of ^{13}C-NMR.

Enzymatic hydrolysis[20] of compound **1** with enzyme takadiastase liberated L-rhamnose (R_f 0.37) and proaglycone, identified as 3β, 28-dihydroxy-20(29)-lupene-3-O-β-D-galactopyranosyl-(1→4)-O-β-D-xylopyranoside, confirming the presence of α-linkage between L-rhamnose and proaglycone. Proaglycone on further hydrolysis with enzyme almond emulsion liberated D-galactose (R_f 0.19) followed by D-xylose (R_f 0.26) and aglycone, suggesting the presence of β-linkage between D-galactose and D-xylose as well as between D-xylose and aglycone.

On the basis of above evidences, the structure of compound **1** was characterized as 3β, 28-dihydroxy-20(29)-lupene-3-O-β-D-galactopyranosyl-(1→4)-O-β-D-xylopyranosyl-28-O-α-L-rhamnopyranoside.

The results reported in **Table I** show that compound **1** was found to be strong active against *Candida albicans* and *Penicillium digitatum* and less active against *Aspergillus niger* and *Tricoderma viride* even at very dilute concentrations. The investigation thus reveals that compound **1** may be potentially useful for diseases caused by these fungi.

Compound **2**, has m.p. 280-283°C, m.f. $C_{30}H_{48}O_3$, $[M]^+$ 456 (EIMS). It was characterized as ursolic acid by comparision of its spectral data with reported literature values.[21-23]

Compound **3**, has m.p. 212-214°C, m.f. $C_{32}H_{52}O_2$, $[M]^+$ 452 (EIMS). It was identified as α-amyrine acetate by comparision of its spectral data with reported literature values [24-25].

Compound **4**, has m.p. 218-219°C, m.f. $C_{30}H_{50}O$, $[M]^+$ 426 (EIMS). It was identified as lupeol by comparision of its spectral data with reported literature values.[26-28]

Experimental Section

Plant material

The leaves of the plant were collected locally around sidhi region and were taxonomically authenticated by taxonomist, Department of Botany, Govt S G S College Sidhi (M.P.) India. A voucher specimen has been deposited in the Laboratory, Department of Chemistry of this college.

General experimental procedure

All of the melting points were determined on a thermoelectrical melting point apparatus and are uncorrected. The IR spectra were recorded in KBr disc on FT-IR spectrometer Shimadzu 8201 PC (4000-400 cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded using solvent $CDCl_3$ and TMS as internal standard on Bruker DRX-300 spectrometer.

Extraction and isolation

Air dried powdered plant leaves (3.6kg) were extracted with 90% ethanol (50-60°C) in a Soxhlet apparatus for 73 hrs. The ethanolic extract was further exhaustively partitioned with chloroform, ethyl acetate, acetone and methanol. The ethyl acetate soluble fraction was further concentrated under reduced pressure to yield brown viscous mass (3.65gm), which was subjected to TLC examination using $CHCl_3$: MeOH: H_2O (26:14:3) as eluent and Liebermann-Burchard reagent as visualizing agent. It gave four spots indicating it to be a mixture of compounds **1**, **2, 3** and **4**. These compounds were separated by TLC and purified by column chromatography over silica gel using solvent gradient system in order to increase polarity i.e. CH_3OAc: MeOH (95:5 to 70:30) as eluent and studied separately.

Study of compound 1

It was crystalysed from mixture of methanol and acetone to yield 1.20 gm. It has m.p. 224-226^{O}C, m.f. $C_{47}H_{78}O_{15}$, $[M]^+$ 882 (FABMS); found(%): C 62.76, H 7.90, O 29.34 calcd.(%) for m.f. $C_{47}H_{78}O_{15}$: C 63.95, H 8.84, O 27.21; UV: λ_{max} nm: (MeOH) 208, 288; IR: ν_{max}^{KBr} (cm^{-1}), 3354 (-OH), 2933, 2862(C-H), 1688(C=C stretching), 1577, 1453, 1368, 1285, 1029, 882,

747; ^{1}H-NMR (500 MHz, $CDCl_3$): δ(ppm): 3.16 (1H, dd, *J* 9.8, 5.2 Hz, H-3α), 0.90 (3H, s, Me-23), 0.71 (3H, s, Me-24), 0.74 (3H, s, Me-25), 0.93 (3H, s, Me-26), 0.95 (3H, s, Me-27), 3.30 (1H, d, *J* 10.7 Hz, H-28_a), 3.76 (1H, d, *J* 10.7 Hz, H-28_b), 4.68 (1H, brs, H-29_a), 4.55 (1H, brs, H-29_b), 1.64 (3H, s, Me-30), 4.78 (1H, d, *J* 7.4Hz, H-1′), 4.05 (1H, m, H-2′), 4.14 (1H, m, H-3′), 4.10 (1H, m, H-4′), 3.70 (1H, d, *J* 10.2Hz, H-5_a), 4.33 (1H, m, H-5_b), 5.10 (1H, d, *J* 7.5Hz, H-1″), 4.41 (1H, m, H-2″), 3.98 (1H, m, H-3″), 4.46 (1H, m, H-4″), 3.97 (1H, m, H-5″), 4.42 (1H, m, H-6″), 5.03 (1H, d, *J* 1.6Hz, H-1‴), 3.86 (1H, d, *J* 2.8,1.6 Hz, H-2‴), 3.85 (1H, dd, *J* 3.2, 9.2 Hz, H-3‴), 3.58 (1H, m, H-4‴), 3.68 (1H, m, H-5‴), 1.30 (3H, *J* 6.4 Hz, Me-6‴). ^{13}C-NMR (125 MHz, $CDCl_3$): δ(ppm): 38.3(C-1), 27.0(C-2), 79.3(C-3), 38.7(C-4), 55.0(C-5), 18.3(C-6), 34.1(C-7), 41.1(C-8), 49.8(C-9), 36.9(C-10), 20.5(C-11), 25.3(C-12), 37.1(C-13), 42.2(C-14), 27.0(C-15), 29.2(C-16), 47.3(C-17), 49.4(C-18), 48.2(C-19), 150.4(C-20), 29.2(C-21), 34.1(C-22), 28.0(C-23), 14.8(C-24), 16.1(C-25), 15.6(C-26), 14.3(C-27), 60.4(C-28), 109.5(C-29), 19.3(C-30), 107.3(C-1′), 74.4(C-2′), 88.3(C-3′), 69.8(C-4′), 66.2(C-5′), 106.3(C-1″), 74.6(C-2″), 75.1(C-3″), 70.4(C-4″), 77.1(C-5″), 62.3(C-6″), 102.2(C-1‴), 71.2(C-2‴), 72.3(C-3‴), 83.1(C-4‴), 69.2(C-5‴), 18.4(C-6‴) and $[M]^+$ 882(FABMS).

Acid hydrolysis of compound 1

Compound **1** (510 mg) was dissolved in ethanol (30 ml) and refluxed with 18 ml of H_2SO_4 on water bath for 9 hrs. The reaction mixture was concentrated and allowed to cool and residue was extracted with diethyl ether (Et_2O). The ether layer was washed with water and evaporated to dryness. The residue was subjected to column chromatography over silica gel column using $CHCl_3$: MeOH (4:8) to give compound **1-A**, identified as 3β, 28-dihydroxy-20(29)-lupene by comparison of its spectral data with reported literature values. The aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination using n-butanol : glacial acetic acid : water (40:10:50) solvent and aniline hydrogen phthalate as spraying reagent, showed the presence of L-rhamnose (R_f0.36), D-xylose (R_f0.28) and D-galactose (R_f0.16)(Co-PC and Co-TLC).

Study of compound 1-A

It has m.f. $C_{30}H_{50}O_2$, m.p. 247-248°C, $[M]^+$ 442 (EIMS); found(%): C 82.90, H 10.86, O 6.24, calcd (%) for m.f. $C_{30}H_{50}O_2$, C 81.45, H 11.31, O 7.24; UV: λ_{max} nm: (MeOH) 212, 292; IR: ν_{max}^{KBr} (cm^{-1}), 3356 (-OH), 2936, 2866(C-H), 1690(C=C stretching), 1578, 1456,

1372, 1288(C-O stretching due to CH_2OH), 1032, 885, 750; ^{1}H-NMR (500 MHz, $CDCl_3$) : δ (ppm): 3.12 (1H, dd, *J* 11.2, 5.0 Hz, H-3), 2.22(1H, dt, *J* 11.2, 6.4 Hz, H-19), 4.68(s, 1H, 3-OH), 4.61(s, 1H, 28-OH), 0.93 (3H, s, Me-23), 0.72(3H, s, Me-24), 0.78(3H, s, Me-25), 0.95(3H, s, Me-26), 0.97(3H, s, Me-27), 3.33(1H, d, *J* 10.8 Hz, H-28_a), 3.79(1H, d, *J* 10.8 Hz, H-28_b), 4.70(1H, brs, H-29_a), 4.58 (1H, brs, H-29_b), 1.66 (3H, s, Me-30). ^{13}C-NMR (125 MHz, $CDCl_3$), δ(ppm): 38.6(C-1), 27.2(C-2), 79.5(C-3), 38.9(C-4), 55.3(C-5), 18.2(C-6), 34.3(C-7), 41.4(C-8), 50.1(C-9), 37.2(C-10), 20.6(C-11), 25.5(C-12), 37.4(C-13), 42.5(C-14), 27.3(C-15), 29.4(C-16), 47.6(C-17), 49.7(C-18), 48.5(C-19), 150.6(C-20), 29.5(C-21), 34.3(C-22), 28.3(C-23), 15.1(C-24), 16.4(C-25), 15.9(C-26), 14.6(C-27), 60.7(C-28), 109.7(C-29), 19.5(C-30).

Permethylation of compound 1

Compound **1** (34mg) was refluxed with MeI (8ml) and Ag_2O (22ml) in DMF (22 mg) for 72 hrs days. The reaction mixture was filtered and washed with DMF. The filtrate was concentrated under reduced pressure and treated with $CHCl_3$ (22ml) and washed with water. After removal of solvent a syrupy mass was obtained which was hydrolyzed with 10% ethanolic H_2SO_4 (15ml) for 7-8 hrs, to give aglycone identified as 3β, 28-dihydroxy-20(29)-lupene. The aqeous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination on Whatmann filter paper No.1 using n-butanol:ethanol:water (5:1:4) solvent and aniline hydrogen phthalate as spraying agent. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.02), 2, 3, 4, 6-tetra-O-methyl-D-galactose (R_G 0.88), 2, 3-di-O-methyl-D-xylose (R_G 0.72).

Enzymatic hydrolysis of compound 1

Compound **1** (26 mg) was dissolved in MeOH (14 ml) and hydrolysed with equal volume of takadiastase enzyme. The reaction mixture was allowed to stay at room temperature for 44 hrs and filtered. The proaglycone and hydrolysate were studied separately. The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent and aniline hydrogen phthalate as spraying reagent, which showed the presence of L-rhamnose (R_f 0.36). The proaglycone was dissolved in MeOH (25 ml) and further hydrolysed with equal volume of almond emulsion enzyme at room temperature as usual procedure yielded aglycone and sugars were identified as D-xylose (R_f 0.28) and D-galactose (R_f 0.16).

Study of compound 2

It has m.f. $C_{30}H_{48}O_3$, m.p. 280-283°C, $[M]^+$ 456 (EIMS); found (%), C 78.52, H 9.90, O 11.58, calcd (%) for m.f. $C_{30}H_{48}O_3$, C 78.95, H 10.53, O 10.53; UV: λ_{max} nm: (MeOH) 472, 443 and 420; IR: ν_{max}^{KBr} (cm^{-1}), 3436(O-H), 2935(COOH stretching), 1746(C=O), 1592(C=C), 1462, 1380, 1350, 962 and 800. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ(ppm) 3.20(1H, dd, *J* 9.2, 6.3 Hz, H-3), 5.23(1H, m, H-12), 2.17(1H, *J* 11.2 Hz, H-19), 0.86(3H, S, Me-23), 0.76(3H, s, Me-24), 0.75(3H, s,Me- 25), 0.84(3H, S, Me-26), 1.02(3H, s, Me-27), 0.82(3H, d, *J* 5.1 Hz, Me-29), 0.92(3H, d, *J* 8.2 Hz Me-30). ^{13}C-NMR (75 MHz, $CDCl_3$) : δ(ppm) 38.16(C-1), 27.84(C-2), 79.63(C-3), 38.16(C-4), 54.43(C-5), 17.87(C-6), 31.4(C-7), 39.80(C-8), 48.16(C-9), 34.31(C-10), 21.63(C-11), 126.84(C-12), 139.74(C-13), 40.71(C-14), 28.51(C-15), 24.12(C-16), 43.27(C-17), 54.45(C-18), 39.79(C-19), 39.89(C-20), 30.72(C-21), 34.35(C-22), 27.82(C-23), 16.12(C-24), 16.34(C-25), 17.62(C-26), 24.13(C-27), 182.03(C-28), 17.85(C-29), 21.63(C-30).

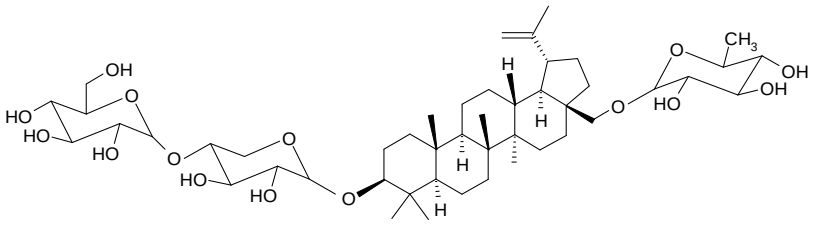
Study of compound 3

It has m.f. $C_{32}H_{52}O_2$, m.p. 212-214°C, $[M]^+$ 452 (EIMS); found (%), C 83.62, H 12.42, O 3.96, calcd(%) for m.f. $C_{32}H_{52}O_2$, C 84.96, H 11.50, O 3.54; IR : ν_{max}^{KBr} (cm^{-1}), 2926, 2850(C-H stretching), 1736(C=O ester), 1446, 1377(CH3 bending), 1240, 1025, 1002, 980, 965, 906, 658, 602. ^{1}H-NMR (400 MHz, $CDCl_3$): δ(ppm) 4.52(1H, dd, *J* 6.2, 9.8 Hz, H-3), 5.15(1H, t, *J* 3.8 Hz, H-12), 0.85(12H, s, Me-23, Me-24, Me-25, Me-26), 1.08(3H, s, Me-27), 0.81(3H, s, Me-28), 1.03(3H, s, Me-29), 0.97(3H, s, Me-30), 2.08(3H, s, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$): δ(ppm) δ 38.6(C-1), 23.8(C-2), 80.6(C-3), 37.4(C-4), 55.1(C-5), 18.6(C-6), 32.5(C-7), 40.3(C-8), 47.9(C-9), 36.5(C-10), 23.8(C11), 124.7(C-12), 139.3(C-13), 42.6(C14), 28.5(C-15), 26.1(C-16), 33.4(C-17), 59.5(C-18), 39.8(C-19), 39.3(C-20), 31.7(C-21), 41.8(C-22), 28.4(C-23), 17.1(C-24), 15.9(C-25), 16.3(C-26), 23.3(C-27), 28.2(C-28), 17.3(C29), 21.6(C-30), 21.8(CO-Acetate), 171.5(CH_3-Acetate).

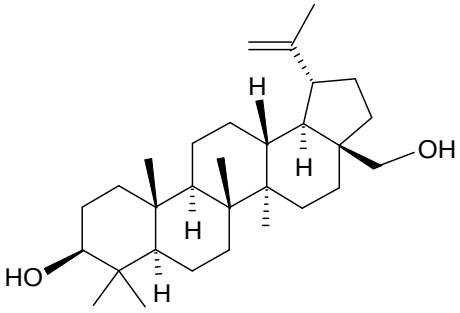
Study of compound 4

It has m.f. $C_{30}H_{50}O$, m.p. 218-219°C, $[M]^+$ 426 (EIMS); found (%), C 84.98, H 11.12, O 3.9, calcd (%) for m.f. $C_{30}H_{50}O$, C 84.50, H 11.74, O 3.76; UV: λ_{max} nm: (MeOH) 340, 292; IR: ν_{max}^{KBr} (cm^{-1}), 3340(O-H), 2946(aliphatic hydrocarbons C-H), 1638(C=C) , 1458, 1382, 1044. ^{1}H-NMR (300 MHz, $CDCl_3$): δ(ppm) 3.18(1H, dd, *J* 4.75, 11.2 Hz, H-3), 4.66(1H, s, H-29_a), 4.58(1H, s, H-29_b), 0.76, 0.79, 0.80, 0.94, 0.97, 1.04, 1.27 (each 3H, s, Me×7). ^{13}C-NMR (75

MHz, $CDCl_3$) : δ(ppm) 38.5(C-1), 25.6(C-2), 79.4(C-3), 38.6(C-4), 55.7(C-5), 18.3(C-6), 34.4(C-7), 41.2(C-8), 50.4(C-9), 37.6(C-10), 21.3(C-11), 27.7(C-12), 39.4(C-13), 43.2(C-14), 27.8(C-15), 35.3(C-16), 43.4(C-17), 48.8(C-18), 48.3(C-19), 151.3(C-20), 30.1(C-21), 40.0(C-22), 28.4(C-23), 15.7(C-24), 16.6(C-25), 16.3(C-26), 14.5(C-27), 18.5(C-28), 109.3(C-29), 19.4(C-30).



Compound 1



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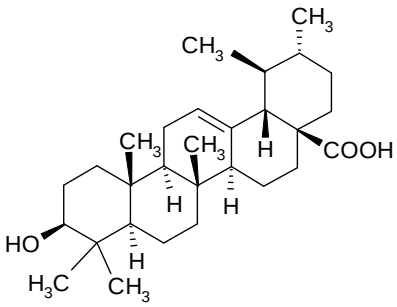

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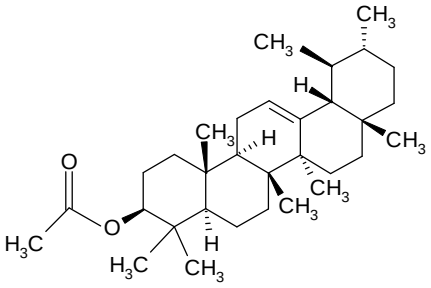

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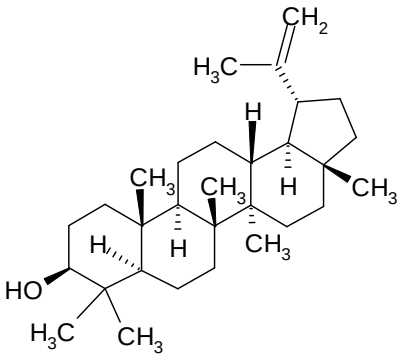

Compound 4

Antifungal activity of compound 1

The antifungal activity of compound **1** was determined by Filter Paper Disc Diffusion method [29-30]. The various fungal species were first incubated at 44°C for 42 hr. The sterile filter paper discs (10 mm) were soaked with standard antifungal agent and various test samples and were dried at 44°C. The discs were then placed on soft nutrient agar (2%) petri plates previously seeded with suspension of each fungal species. The diameters of zone of inhibition were measured at 40±1°C after 25 hr. The results are recorded in **Table I**.

Table I: Antifungal activity of compound 1.

Sr.No.	Fungal species	Diameters of zone of inhibition (mm)* Concentration of compound 1 (%)				Std.**
		100	80	60	40	
1.	*Aspergillus niger*	12.5	7.8	5.7	4.3	18.8
3.	*Candida albicans*	18.2	15.9	12.1	10.2	22.3
2.	*Trichoderma viride*	11.4	8.3	5.6	1.7	15.6
4.	*Penicillium digitatum*	16.9	14.1	10.5	8.3	22.8

*The zone of inhibition (mm) taken as average of four determination direction.

**Ketocozole (100 mg/mL) used as standard antifungal agent.

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