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New antifungal triterpenoid saponin from *Launaea pinnatifida* Cass.

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A new triterpenoid saponin (compound **1**), 3β-O-[α-L-rhamnopyranosyl-(1→3)-O-α-L-arabinopyranosyl-(1→3)-O-β-D-galactopyranosyl]-spergulatriol along with known compounds glutenol and hopenol-b have been isolated from the methanol soluble fraction of the defatted seeds of the plant and structurally elucidated by various colour reactions, chemical degradations and spectral analysis. Compound **1** shows antifungal activity against various fungi.

Keywords: *Launaea pinnatifida* Cass., Compositae, seeds, triterpenoid saponin, antifungal activity

Launaea pinnatifida Cass. which is commonly known as "Bankau" in Hindi belongs to family Compositae. It is distributed in sandy coasts of India, Sri Lanka, Malaysia and East Africa. It is reported to possess tonic, soporific, diuretic and aperient properties[1-4]. Earlier workers[5] have reported various constituents from this plant. In the present paper is reported the isolation and structural elucidation of a new triterpenoid saponin 3β-O-[α-L-rhamnopyranosyl-(1→3)-O-α-L-arabinopyranosyl-(1→3)-O-β-D-galactopyranosyl]-spergulatriol and known compounds glutenol and hopenol-b from the seeds of this plant.

Results and Discussion

The methanolic extract of the seeds of the plant afforded a new compound **1**, m.p. 274-76°C, Mol. formula $C_{45}H_{74}O_{16,}$ $[M]^+$; *m/z* 870 (FABMS). It gave positive test for triterpenoid saponins[6-9]. It also gave positive Molisch test. In ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of compound **1**, a double doublet at δ 0.84 (dd, *J* = 2.5, 12.3 Hz) and a doublet at δ 1.76 (d, *J* = 10.5 Hz) were assigned to H-5α and H-13β respectively. Six signals at δ 1.28, 1.06, 0.86, 1.05, 1.14, and 1.07 were assigned for six methyl groups at C-23, C-24, C-25, C-26, C-27, C-28 . Three doublets at δ 4.78 (d, *J*=2.0 Hz), δ 4.95 (d, *J* =2.0 Hz), δ 4.94 (d, *J*=7.8 Hz) were assigned to H-1', H-1" and H-1'" of D-galactose, L-arabinose, L-rhamnose respectively. In the mass spectrum of compound **1** characteristic ions at *m/z* 723 $[M-H-146]^+$, 591$[M-H-146-132]^+$, 429 $[M-H-146-132-162]^+$ were found by subsequent losses of one molecule of L-rhamnose, L-arabinose and D-galactose units each suggesting L-rhamnose as the terminal sugar, L-arabinose as the middle sugar and D-galactose was directly attached to -OH group at C-3 position in compound **5**.

The sequence of the sugar residue in compound **1** was confirmed by its partial hydrolysis[10] with Killiani mixture [HCl:AcOH:H_2O (15:35:50)] which gave compound **6** and L-rhamnose. Compound **6** on further hydrolysis gave compound **7** and L-arabinose. compound **7** on further hydrolysis gave compound **5** and D-galactose.

Acid hydrolysis of compound **1** with 2 N H_2SO_4 gave compound **5** m.p. 222-24°C, Mol. formula $C_{28}H_{46}O_3$, $[M]^+$; 430 (FABMS) identified as spergulatriol[11].

The aqueous hydrolysate obtained after acid hydrolysis of the compound **1** was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination using *n*-BAW (4:1:5) as eluent and aniline hydrogen phthalate as detecting agent yielded D-galactose (R_f 0.15), L-rhamnose (R_f 0.35) and L-arabinose (R_f 0.20) (Co-PC)[12].

Permethylation[13] of compound **1** followed by acid hydrolysis yielded permethylated aglycone identified as 3β-hydroxy, 12,16 dimethoxy, spergulatriol and permethylated sugars which were identified as 2,3,4-tri-O-methyl-L-rhamnose, 2,4-di-O-methyl-L-arabinose and 2,4,6-tri-O-methyl-D-galactose (by Co-PC) according to Petek suggesting that C-1'"

of L-rhamnose was attached with C-3" of L-arabinose and C-1" of L-arabinose was linked with C-3' of D-galactose. The interlinkage (1→3) between the sugars were further confirmed by its ^{13}C NMR spectral data (see Experimental Section). Periodate oxidation[14] of compound **1** confirmed that three sugars were present in pyranose form. Compound **1** when treated with sodium meta-periodate consumed 3.21 moles of periodate and liberated 1.04 moles of formic acid, suggesting the presence of trisaccharide nature of sugar unit and also indicating that all the sugars were present in pyranose form.

Enzymatic hydrolysis[15] of compound **1** with enzyme takadiastase liberated L-rhamnose (R_f 0.35) first followed by L-arabinose (R_f 0.20) (by Co-PC) and proaglycone (compound **7**) identified as 3β-O-[β-D-galactopyranosyl]-spergulatriol (see Experimental Section) suggesting the presence of α-linkage between L-rhamnose and L-arabinose as well as between L-arabinose (R_f 0.20) and D-galactose. Proaglycone (compound **7**) was hydrolysed with enzyme almond emulsin liberated D-galactose (R_f 0.15) and sapogenin (aglycone) revealing the presence of β-linkage between D-galactose and sapogenin.

On the basis of above evidence the structure of compound **1** was established as 3β-O-[α-L-rhamnopyranosyl-(1→3)-O-α-L-arabinopyranosyl-(1→3)-O-β-D-galactopyranosyl]-spergulatriol.

Compound **1** was tested against various fungi. The results obtained in **Table I** showed that the antifungal activity of compound **1** is fairly good against *Penicillium notatum* and *Fusarium oxysporum* even in very low concentrations.

Experimental Section

All the melting points were determined by Thiele apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer 1800 FTIR spectrometer. ^1H NMR spectra were recorded at 300 MHz on Bruker DRX 300 NMR spectrometer using TMS as internal standard and $CDCl_3$ as solvent. ^{13}C NMR were recorded at 90 MHz and DMSO-d_6 as solvent and mass spectra on a Jeol SX-102 (FABMS) mass spectrometer.

Plant material

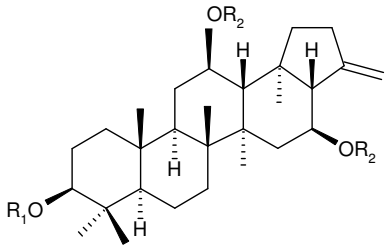
The seeds of *Launaea pinnatifida* Cass were procured from M/s United Chemicals and Allied Products, Kolkata and were taxonomically authenticated by the Chief Taxonomist, Department of Botany, Dr. H.S. Gour University Sagar (M.P.) India.

Extraction and Isolation

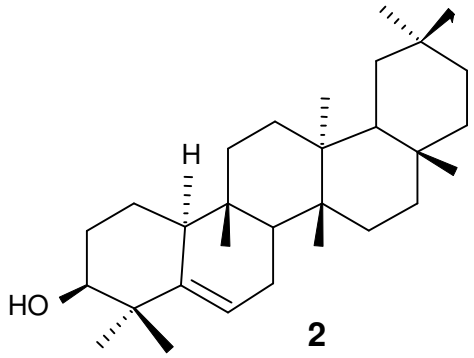
Dried powdered seeds (3 kg) of the plant were extracted with petroleum ether (40-60°C) in a Soxhlet extractor. The defatted seeds of the plant were further extracted with 95% methanol. The total ethanolic extract was concentrated under reduced pressure to yield light brown coloured mass. It gave four spots on TLC examination using chloroform:ethanol:water (6:4:2) as eluant and I_2 vapours as visualizing agent indicating it to be a mixture of compounds **1, 2, 3** and **4** . These were separated by column chromatography over a silica-gel column using $CHCl_3$:MeOH (3:6) as eluant again yielded compounds **1, 2, 3** and **4** which were further purified by preparative TLC. Compound **4** was isolated in very small quantity therefore it was not possible to examine it further. Compound **1** was purified by recrystallization from methanol to yield light yellowish needles (1.25 g).

Study of compound 1

It had m.p. 274-76°C; Mol. formula $C_{45}H_{74}O_{16}$; FABMS: *m/z* 870 $[M]^+$. Anal. Found C, 62.04; H, 8.47. Calcd for $C_{45}H_{74}O_{16}$: C, 62.06; H, 8.50%. IR (KBr): 3430, 1650, 1084, 1032, 965 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.20 (1H, dd, *J*=4.2, 11.6 Hz, H-3α), 0.84 (1H, dd, *J*=2.5, 12.3 Hz, H-5α), 1.46 (1H, m, H-9α), 4.21 (1H, m, H-12α), 1.76 (1H, d, *J* =10.5 Hz, H-13β), 4.30 (1H, dd, *J*=4.4, 10.1 Hz, H-16α), 2.10 (1H, m, H-17 β), δ1.28, 1.06, 0.86, 1.05, 1.14, 1.07 (6 × CH_3, s, C-23, C-24, 25, 26, 27, 28), 5.14 (2H, t, *J*=2.1 Hz, H-22), 4.78 (1H, d, *J*=2.1 Hz, H-1'), 3.41 (1H, dd, *J*=7.1, 8.5 Hz, H-2'), 3.51 (1H, dd, *J*= 8.3, 4.2 Hz, H-3'), 3.85 (1H, dd, *J*=4.0, 2.6 Hz, H-4'), 3.51 (1H, m, H-5'), 3.86 (2H, dd, *J*= 2.3, 4.5 Hz, H-6'), 4.95 (1H, d, *J*=2.1 Hz, H-1"), 4.12-4.23 (3H, m, H-2", 3", 4"), 4.31 (1H, m, H-5"), 4.94 (1H, d, *J*=7.8 Hz, H-1'"), 4.83 (1H, d, *J*=2.4 Hz, H-2'"), 4.65 (1H, dd, *J*= 3.2, 9.6 Hz, H-3'"), 4.31 (1H, m, H-4'"), 4.78 (m, H-5'"), 1.05 (3H, d, *J*=6.1 Hz, rhamnosyl methyl); ^{13}C NMR (90 MHz, DMSO-d_6): δ 40.2 (C-1), 28.1 (C-2), 78.3 (C-3), 39.6 (C-4), 55.6 (C-5), 18.8 (C-6), 33.7 (C-7), 45.4 (C-8), 49.6 (C-9), 37.4 (C-10), 33.4 (C-11), 69.4 (C-12), 53.8 (C-13), 41.7 (C-14), 45.3 (C-15), 67.3 (C-16), 62.1 (C-17), 45.36 (C-18), 43.3 (C-19), 29.8 (C-20), 151.6 (C-21), 106.3 (C-22), 28.4 (C-23), 16.5 (C-24), 16.2 (C-25), 17.2 (C-26), 19.3 (C-27), 16.1 (C-28), 101.6 (C-1'), 71.1 (C-2'), 73.0 (C-3'), 68.3 (C-4'), 73.8 (C-5'), 65.6 (C-6'), 106.03 (C-1"), 76.0 (C-2"), 77.6 (C-3"), 70.2 (C-4"), 66.3 (C-5"), 101.2 (C-1'"), 71.4 (C-2'"), 70.4 (C-3'"), 73.8 (C-4'"), 69.6 (C-5'"), 18.7 (C-6'", rhamnosyl methyl); FABMS: *m/z* 871, 870, 869, 723, 591, 431,430, 429, 413, 377.



Compd	R_1	R_2
1	rham(1→3)-arab(1→3)-gal	H
5	H	H
6	arab(1→3)-gal	H
7	gal	H
8	rham(1→3)-arab(1→3)-octa methylate	Me
9	arab(1→3)-gal-hexa methylate	Me
10	Gal tetra methylate	Me



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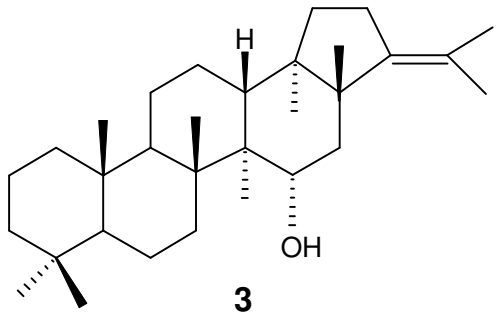

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Acid hydrolysis of compound 1

Compound **1** (500 mg) was dissolved in ethanol (25 mL) and refluxed with 10% methanolic H_2SO_4 on water bath for 8 hr. The reaction mixture was concentrated and allowed to cool and the residue was treated with Et_2O. The ethereal layer was washed and evaporated to dryness. The residue was subjected to column chromatography over a silica-gel column using $CHCl_3$:MeOH (6:8) to give compound **5**. It responded to all the colour reactions of triterpenoids[16-18] and identified as spergulatriol by comparison of its spectral data (IR, ^{1}H NMR, MS, ^{13}C NMR), with reported literature values. The aqueous hydrolysate was neutralized with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination using (*n*-BAW, 4:1:5) as solvent and aniline hydrogen phthalate as spraying reagent to show the presence of L-arabinose (R_f 0.20), L-rhamnose (R_f 0.35) and D-galactose (R_f 0.15) (Co-PC).

Study of compound 5

It had m.p. 222-24°C; Mol. formula $C_{28}H_{46}O_3$; FABMS: *m/z* 430 $[M]^+$. Anal. Found C, 74.08; H, 10.16. Calcd for $C_{28}H_{46}O_3$: C, 74.09; H, 10.13%. IR(KBr): 3435, 1664, 1082, 1039, 973 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.18(1H, dd, *J*=4.1, 11.4 Hz, H-3α), 0.84 (1H, dd, *J*=2.5, 12.3 Hz, H-5α), 1.45 (1H, m, H-9α), 4.26 (1H, m, H-12α), 1.78 (1H, d, *J*= 11.0 Hz, H-13β), 4.30 (1H, dd, *J*=4.4, 10.1 Hz, H-16α), 2.11 (1H, m, H-17 β), 5.16 (2H, t, *J*=2.2 Hz, H-22), 0.87-1.26 (6 × CH_3 , s, C-23, 24, 25, 26,27, 28).

Partial hydrolysis of compound 1

Compound **1** (150 mg) was treated with Killiani mixture [50 mL, HCl:AcOH:H_2O (15:35:50)] and stirred at RT for 8 hr. The reaction mixture was then treated with *n*-BuOH to give compound **6** (50 mg) and L-rhamnose (R_f 0.35). Compound **6** on further hydrolysis gave compound **7** identified as 3β-O-[β-D-galactopyranosyl]-spergulatriol and L-arabinose (R_f 0.20)

Table I — Antifungal activity of compound **1**

	Diameter of zone of inhibition (mm)* Compound **1** at concentrations %					Std**
Fungal species	100%	80%	60%	40%	20%	100%
Penicillium notatum	10.4	8.5	6.2	4.8	3.1	12.5
Aspergillus fumigatus	7.4	5.6	3.2	1.3	-	13.5
Fusarium oxysporum	9.8	7.4	5.3	3.8	2.5	12.0
Trichoderma viride	10.6	5.4	2.5	1.00	-	23.00

* The diameters of zone of inhibition (mm) taken in different directions
** Griseofulvin (1000 ppm) used as standard antifungal agent.

Study of compound 6

It had m.p. 273-75°C, Mol. formula $C_{39}H_{64}O_{12}$; FABMS: *m/z* 724 $[M]^+$. Anal. Found C, 64.62; H, 8.82. Calcd for $C_{39}H_{64}O_{12}$: C, 64.64; H, 8.83%. IR(KBr): 3436, 1658, 1078, 1031, 975 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.27, 1.07, 0.84, 1.04, 1.15, 1.05 (each 3H, s, for C-23, C-24, C-25, C-26, C-27, C-28), 3.46 (1H, m, H-3α), 0.76 (1H, dd, *J*=2.1, 12.4 Hz, H-5α), 1.46 (1H, m, H-9α), 4.20 (m, H-12 α), 1.72 (1H, d, *J*=10.6 Hz, H-13 β), 2.12 (1H, m, H-17 β), 5.17 (2H, t, *J*=2.3 Hz, H-22), 5.48 (1H, d, *J*=7.9 Hz, H-1'), 3.43 (1H, dd, *J* =7.2, 8.6 Hz, H-2'), 3.53 (1H, dd, *J*=8.4, 4.3 Hz, H-3'), 3.86 (1H, dd, *J*= 4.2 , 2.8 Hz, H-4'), 3.54 (1H, m, H-5'), 3.87 (2H, dd, *J*=2.2, 4.7 Hz, H-6'), 4.97 (1H, d, *J*=2.3 Hz, H-1"), 4.25 (1H, m, H-2"), 4.18 (1H, m, H-3"), 4.16 (1H, m, H-4"), 4.36 (m, H_a-5"), 3.74 (1H, dd, *J*=9.2, 11.4 Hz, H_b-5"); ^{13}C NMR (90 MHz, DMSO-d_6): δ 39.6 (C-1), 28.5 (C-2), 78.4 (C-3), 39.6 (C-4), 55.8 (C-5), 18.7 (C-6), 33.7 (C-7), 45.5 (C-8), 49.7 (C-9), 37.4 (C-10), 33.4 (C-11), 69.7 (C-12), 54.1(C-13), 42.2 (C-14), 45.2 (C-15), 67.8 (C-16), 62.4 (C-17), 45.41 (C-18), 43.1 (C-19), 30.1 (C-20), 151.9 (C-21), 106.5 (C-22), 28.3 (C-23), 16.8 (C-24), 16.2 (C-25), 17.2 (C-26), 19.3 (C-27), 16.3 (C-28), 101.7 (C-1'), 74.9 (C-2'), 78.31 (C-3'), 71.8 (C-4'), 77.2 (C-5'), 62.1 (C-6'), 106.7 (C-1"), 71.4 (C-2"), 74.2 (C-3"), 70.8 (C-4"), 69.9 (C-5"). It was identified as 3β-O-[α-L-arabinopyranosyl-(1→3)-O-β-D-galactopyranosyl]-spergulatriol.

Study of compound 7

It had m.p. 262-64°C, Mol. formula $C_{34}H_{56}O_8$; FABMS: *m/z* 592 $[M]^+$. Anal. Found C, 68.89; H, 9.46. Calcd for $C_{34}H_{56}O_8$: C, 68.91; H, 9.45%. IR(KBr): 3436, 1668, 1085, 974 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.28, 1.21, 0.84, 1.03, 1.09, 1.07 (each 3H, s, for C-23, C-24, C-25, C-26, C-27, C-28), 3.46 (1H, m, H-3α), 0.78 (1H, dd, *J*=2.2, 12.3 Hz, H-5α), 1.46 (1H, m, H-9α), 4.19 (1H, m, H-12 α), 1.75 (1H, d, *J*=10.4 Hz, H-13β), 2.11 (1H, m, H-17β), 4.75 (1H, d, *J*=10.1 Hz, H-1'), 3.41 (1H, dd, *J*=7.1, 8.5 Hz, H-2'), 3.52 (1H, dd, *J*=8.1, 4.2 Hz, H-3'), 3.84 (1H, dd, *J*=4.3, 2.7 z, H-4'), 3.52 (1H, m, H-5'), 3.88 (2H, dd, *J*= 2.5, 4.4 Hz, H-6').

Permethylation followed by hydrolysis of compound 1

Compound **1, 6** and **7** (10 mg each) separately in MeI (5 mL) and Ag_2O (20 mg) in DMF (6 mL) were treated at RT for one day. The total reaction-mixture were diluted with water and extracted with $CHCl_3$ (20 mL) to yield compounds **8**, **9** and **10** respectively. Compounds **8, 9** and **10** were hydrolysed with Killiani mixture separately and filtered. The filtrates of each were neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and examined by PC using *n*-BuOH:AcOH:H_2O (4:1:5) as solvents. The methylated sugars were identified as 2,3,4-tri-O-methyl–L-rhamnose, 2,4-di-O-methyl-L-arabinose and 2,4,6-tri-O-methyl–D-galactose for compound **8**, 2,4-di-O-methyl-L-arabinose and 2,4,6-tri-O-methyl-D-galactose for compound **9** and 2,4,6-tri-O-methyl-D-galactose for compound **10**.

Periodate oxidation of compound 1

Compound **1** was dissolved in MeOH and treated with sodium meta periodate for 36 hr. The liberation of formic acid and consumed periodate were estimated by Jone's method.

Enzymatic hydrolysis of compound 1

Compound **1** (15 mg) was dissolved in MeOH (20 mL) and hydrolysed with equal volume of enzyme takadiastase in a 100 mL round bottomed flask fitted with air condenser. The total contents were allowed to stand at RT for 3 days and filtered and studied separately. The hydrolysate was concentrated and subjected to paper chromatography examination using *n*-BAW (4:1:5) as solvent system and aniline hydrogen phthalate as spraying agent gave L-rhamnose (R_f 0.35), and L-arabinose (R_f 0.20), suggesting the presence of α-linkage between L-rhamnose and L-arabinose as well as between L-arabinose and proaglycone (compound **7**). Proaglycone (compound **7**) on further hydrolysis with enzyme almond emulsin yielded D-galactose (R_f 0.15), and aglycone (sapogenin) which confirmed the presence of β-linkage between D-galactose and aglycone.

Study of compound 2

It had m.p. 208-09°C; ^1H NMR (300 MHz, $CDCl_3$): δ 0.85, 0.95, 0.98, 1.00, 1.05, 1.09, 1.15 and 1.17 (each 3H, s, CH_3-23, 24, 25, 26, 27, 28 29, 30), 3.45 (1H, brs, H-3), 5.60 (1H, m, polymethylene CH_2); ^{13}C NMR (90 MHz, DMSO-d_6): δ 39.1 (C-1), 28.0 (C-2), 76.4 (C-3), 39.4 (C-4), 141.7 (C-5), 122.1 (C-6), 33.3 (C-7), 49.9 (C-8), 38.0 (C-9), 47.6 (C-10), 35.2 (C-11), 23.8 (C-12), 31.8 (C-13), 34.9 (C-14), 32.4 (C-15), 30.5 (C-16), 30.2 (C-17), 43.2 (C-18), 36.1 (C-19), 29.8 (C-20), 29.0 (C-21), 34.5 (C-22), 34.7 (C-23), 18.3 (C-24), 19.7 (C-25), 16.3 (C-26),

18.4 (C-27), 28.3 (C-28), 32.2 (C-29), 25.8 (C-30); FABMS: *m/z* 427, 426, 411, 408, 393, 274, 259, 205. It was identified as Glutenol.

Study of compound 3

It had m.p. 252-54°C; IR(KBr): 3615, 1085, 1635, 983 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.72-1.24 (3H, s, 7 × CH_3, C-23, 24, 25, 26, 27, 28, 30), 3.18 (1H, q, *J*=12.0, 6.0 Hz, H-3), 4.76 (2H, br, s, *J*=4.6 Hz, H-29); FABMS: *m/z* 427, 426 ,411, 393, 383, 378, 370, 315, 207, 204, 189. Intense peaks at *m/z* 207 and 189 along with peaks at *m/z* 383 and 370 were due to cleavage of ring C in compound **3** indicating hopane skeleton. It was identified as Hopenol-b [hop-22 (29) ene-3β ol].

Antimicrobial study of compound 1

The antifungal activity of compound **1** was tested against various fungi. A 1000 ppm stock solution of compound **1** was prepared in methanol and diluted to different concentrations. The zone of inhibition were recorded at 26±1°C after 48 hr for fungi.

The antifungal activity was determined by the Filter Paper Discs (6 mm) Method[19,20]. Petri discs were placed on "Sabouraud's broth medium" (1%). The zone of inhibition were expressed as an average of the maximum diameter in four different directions. The various results are shown in **Table I**.

Acknowledgement

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References

1 Kirtikar K R & Basu B D, *Indian Medicinal Plants*, Vol II, (Lalit Mohan Basu and Co, Allahabad), **1999**,1447.
2 *The Wealth of India*, A Dictionary of Raw Materials and Industrial Products, Vol VI, (CSIR Publications, New Delhi), **1988**, 42.
3 Chopra R N, Nayar S L & Chopra I C, *Glossary of Indian Medicinal Plants*, (CSIR Publications, New Delhi), **1996**,150.
4 Nadkarni K M, *Indian Materia Medica*, (Bombay Popular Prakashan, Bombay) **1976**, 728.
5 Prabhu K R & Venkateshwarlu V, *J Indian Chem Soc*, 46 (2), **1969**,146.
6 Kolasapithes E, *Gyogyozerzet*, 4, **1960**.
7 Tschugajew R, *Chem Zig,* 24, **1990**, 542.
8 Sannie C H, *Annual Biochem Med*, 9, **1948**, 175.
9 Brikorn C H & Briner M, *Pharm Acta Helv*, 28, **1953**, 139.
10 Dutta T & Basu H P, *Indian J Chem*, 6, **1968**, 471.
11 Sahu N P, Koike K & Banerjee S, *Phytochemistry*, 58, **2001**, 1177.
12 Lederer E & Lederer M, *Chromatography*, (Elsevier, London) **1957**, 247.
13 Kuhn R, Trischmann H & Low J, *Angew Chem*, 67, **1953**, 32.
14 Hirst E L & Jone J K N, *J Chem Soc*, **1949**, 1659.
15 Saunders B C & Mann F G, *Practical Organic Chemistry*, (Longman, New York), **1936**, 365.
16 Noller C R, Smith R A, Morris G H & Walker J W, *J Am Chem Soc*, **1942**, 64.
17 Salkowaski E, *Hoppe Sevl Z*, 57, **1908**, 521.
18 Liebermann C, *Berdt Chem Soc Ges*, 18, **1885**, 1804.
19 Jasper C, Maruzella J C & Henry PA, *J Am Pharm Assoc*, 47, **1958**, 471.
20 Vincent J C & Vincent H W, *Proc Soc Exp Bio Med*, **1944**, 55.