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Bioactive Compounds from *Acrotrema arnottianum*

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Lupeol, lupeol acetate, *n*-dotriacontanol, β-sitosterol and *n*-triacontanoic acid have been isolated and characterized from hexane extract and kaempferol, quercetin, 3-methoxy-4-hydroxy benzoic acid, quercetin-3-*O*-β-D-rhamnoside and kaempferol-4′-*O*-β-glucopyranoside have been identified from the methanol extract of *Acrotrema arnottianum* (whole plant). Antioxidant activity of the isolated compounds was studied by inhibition of lipid peroxidation in liver homogenate of adult male Wistar rats induced by Fe^{2+}/ascorbate system and quantified spectrometrically. Antimicrobial activity of the extracts and isolates have been studied by disc diffusion technique.

Key Words: *Acrotrema arnottianum*, Hexane and methanol extracts, Chemical constituents, Antioxidant and Antimicrobial activities.

INTRODUCTION

Acrotrema arnottianum (Wight) (Malayalam name "Nilampunna") [Dilleniaceae] is a herb, stems very short or absent, with woody rhizomes. The plant is distributed in the southern Western Ghats of peninsular India, mostly in Kerala and Tamil Nadu. The plant is abundant in moist deciduous and evergreen forests at altitudes up to 800 m[1, 2]. It is used by the village healers and the "Malavedan" tribes. Medicated oil prepared with the whole plant is applied externally over the scalp to prevent hair fall and in combination applied externally against baldness[3]. Fresh leaves are used to relieve headache[4]. In the present communication the isolation and characterization of ten compounds from the hexane and methanol extracts of the shade dried whole plant and their antimicrobial and antioxidant activities have been reported.

EXPERIMENTAL

Whole plant of *A. arnottianum* was collected from Palode (Kerala, India) in January 2001 and identified by Mr. T. Dhruvan, Tropical Botanic Garden and Research Institute (TBGRI), Thiruvananthapuram, Kerala, where a voucher specimen No. 40085 is deposited in the Phytochemistry Division of Tropical Botanic Garden and Research Institute.

1 kg of the shade dried pulverized whole plant was extracted successively with

hexane (4 L, 48 h) and methanol (4 L, 48 h) in a soxhlet to obtain 33 g of hexane and 120 g of methanol extracts. The hexane extract (31 g) was chromatographed on a silica gel (60–120 mesh, 950 g) column (200 × 5 cm) using gradient elution with the solvents hexane, EtOAc and MeOH to afford five compounds designated **Aa-1, Aa-2, Aa-3, Aa-4, Aa-5**. The methanol extract (114 g) was suspended in water, partitioned between EtOAc and *n*-butanol. The EtOAc extract (10.5 g) was chromatographed over silica gel (60–120 mesh) using gradient elution with the solvents hexane, EtOAc and MeOH to get three compounds designated **Aa-6, Aa-7** and **Aa-8**. Butanol extract (7.5 g) on chromatography over silica gel (60–120 mesh) column using gradient elution with the solvents EtOAc and MeOH, followed by MPLC, yielded two compounds designated **Aa-9** and **Aa-10**. The structures of the isolates were elucidated by UV, IR, ^{1}H and ^{13}C NMR and MS data.

The antimicrobial study was carried out by disc diffusion (Kirby-Bauer) technique[5, 6]. This is the first report on the antimicrobial study of the extracts and isolated compounds from *A. arnottianum*. Hexane and methanol extracts were prepared from shade dried, powdered whole plant of *A. arnottianum*. The extracts and the pure compounds isolated from *Acrotrema arnottianum* were used for the study. Both gram positive and gram negative bacteria (Table-1) were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India as microbial type culture collection (MTCC). Filter paper discs of uniform size, impregnated with specified concentration of the different extracts (100 μg/disc), the test compounds (100 μg/disc), the reference streptomycin (2 μg/disc) and control were placed on the surface of the agar plate that had been seeded with the organism. The medium of choice was nutrient-agar with a pH of 7.2–7.4 (Tables 1 and 2).

Antioxidative activity of the isolated compounds from *A. arnottianum* were studied by inhibition of lipid peroxidation in liver homogenate of adult male Wistar rats induced by Fe^{2+}/ascorbate system and quantified spectrometrically at 532 nm by the TBA (thiobarbituric acid) test[7]. Pure compounds isolated from *Acrotrema arnottianum*, *viz.*, **Aa-1** (lupeol acetate), **Aa-3** (dotriacontanol), **Aa-5** (triacontanoic acid), **Aa-6** (kaempferol), **Aa-7** (quercetin), **Aa-8** (vanillic acid), **Aa-9** (quercetin) and **Aa-10** (kaempferol 4′-O-β-D glucopyranoside) were tested (Table-3).

The effect of anti-$FeCl_2$-ascorbic acid stimulated lipid peroxidation of the pure compounds was studied *in vitro*, by adopting the modified method[7]. Male Wistar rats weighing 150 g (12–16 weeks of age) were used. Approximately, 2 g of mouse liver tissue was sliced and then homogenized with 10 mL of 150 mM KCl-tris-HCl buffer (pH 7.2) and centrifuged at 3000 rpm for 10 min to get a supernatant as liver homogenate. A mixture containing 0.25 mL of liver homogenate, 0.1 mL of tris-HCl buffer, 0.05 mL of 0.1 mM ascorbic acid, 0.05 mL of 4 mM $FeCl_2$ and 0.05 mL (20 μg/mL) of the pure compound was shaken well and incubated for 1 h at 37°C. Control groups were incubated without $FeCl_2$-ascorbic acid (NC). Another control group had $FeCl_2$-ascorbic acid but was without test compound (IC). After incubation, 0.5 mL of 0.1 N HCl, 0.2 mL of 9.8% SDS (sodium dodecyl sulphate), and 0.9 mL of distilled water, followed by

2 mL of 0.6% TBA (thiobarbituric acid) were added.. After vigorous shaking the mixture was heated for 30 min. on a boiling water bath. After cooling, 5 mL of *n*-butanol was added and the mixture was again shaken vigorously. The *n*-butanol layer was separated by centrifugation. The absorbance of the supernatant was measured at 532 nm. The malonic dialdehyde (MDA) production was measured and the percentage inhibition was calculated. The results of the estimation of anti-$FeCl_2$-ascorbic acid stimulated lipid peroxidation in rat liver homogenate are shown in Table-3.

RESULTS AND DISCUSSION

Lupeol acetate (Aa-1)

Aa-1, colourless crystals from chloroform/methanol, yield 21 mg (0.002%), m.p. 216°C, tested positive with Liebermann-Burchard reagent for terpenoids. TLC: hexane/$CHCl_3$ (8 : 2), $R_f = 0.58$, $[\alpha]_D = +27°$ ($CHCl_3$). IR (KBr, cm^{-1}): 2939, 2631, 1726, 1633, 1449, 1377, 1363, 1240, 1023, 969, 870. ^1H NMR (400 MHz, $CDCl_3$): δ 4.74 (1H, br s, Hb-29), 4.62 (1H, br s, Ha-29), 4.52 (1H, dd, J = 10 and 5.2 Hz, H-3α), 2.44 (1H, m, H-19), 2.09 (3H, s, Me-CO-O), 1.73 (3H, s, Me-30), 1.08, 0.99, 0.90, 0.89, 0.88, and 0.84 (3H each, s). ^{13}C NMR (100 MHz, $CDCl_3$): ppm 37.7 (C-1), 25.0 (C-2), 80.9 (C-3), 38.3 (C-4), 55.3 (C-5), 18.1 (C-6), 34.1 (C-7), 40.8 (C-8), 50.3 (C-9), 37.0 (C-10), 20.9 (C-11), 23.6 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.5 (C-16), 42.9 (C-17), 48.2 (C-18), 47.9 (C-19), 150.9 (C-20), 29.8 (C-21), 39.9 (C-22), 27.9 (C-23), 15.9 (C-24), 16.4 (C-25), 16.1 (C-26), 14.4 (C-27), 17.9 (C-28), 109.3 (C-29), 19.2 (C-30), 171.0 (C-31), 21.3 (C-32). EI-MS (70 eV) m/z (%): 469 (100), 454 (15) 426 (2), 359 (20), 218 (90), 189 (60), 175 (20), 136 (40), 121 (40)

On alkaline hydrolysis **Aa-1** afforded lupeol (m.p. 214°C). The chemical evidence as well as comparison of IR, MS and NMR spectral data with those reported in literature[8–10] supported the identity of **Aa-1** as lupeol acetate. Further confirmation of the identity of **Aa-1** has been established by m.m.p, co-TLC and superimposable IR spectrum with an authentic sample.

Lup-20(29)-en-3-β-ol (Aa-2)

Aa-2 was obtained as crystalline solid from hexane/benzene, yield 35 mg (0.003%), m.p. 214°C. $[\alpha]_D = +27°$ ($CHCl_3$). It gave a pink colour in the Liebermann-Burchard test for triterpenes. TLC: hexane/$CHCl_3$ (8 : 2), $R_f = 0.50$. IR (KBr, cm^{-1}): 3360, 2921, 2840, 2396, 1638, 1115, 1027, 872. EI-MS (70 eV) m/z: 426, 408, 393, 257, 207, 189, 175, 161.

On refluxing with Ac_2O/pyridine **Aa-2** afforded an acetate, m.p. 216°C. Based on the above data **Aa-2** has been identified as lupeol, (lup-20(29)-en-3-β-ol). The identity was further confirmed by m.m.p, co-TLC, superimposable IR spectrum with an authentic sample and by comparison with literature data[10].

n-Dotriacontanol (Aa-3)

Aa-3 was obtained as colourless flakes from hexane/chloroform, yield 28 mg (0.003%), m.p. 81–82°C. TLC: hexane/EtOAc (8 : 2), $R_f = 0.43$. IR (KBr, cm^{-1}):

3303, 2915, 2848, 1467, 1379, 723. EI-MS (70 eV) m/z (%): 448 (4), 420 (38), 392 (18), 364 (2), 336 (2), 335 (4), 307 (3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (3H, t, J = 6.6, —CH_3) 1.60, 1.52 and 1.25 (50 H, brs, 25 × —CH_2), 3.13 (2H, t, J = 6.6 Hz, CH_2-OH). Comparison of the above data with those reported for *n*-dotriacontanol in the literature[11–14] suggested the identity of **Aa-3** as *n*-dotriacontanol. The identity was further confirmed by m.m.p, co-TLC and superimposable IR spectrum with an authentic sample of *n*-dotriacontanol.

β-Sitosterol (Aa-4)

Aa-4 was obtained as white crystals from hexane/EtOAc, yield 38 mg (0.004%), m.p. 138°C. Gave positive test for steroid with Liebermann-Burchard reagent, TLC: hexane/EtOAc (8 : 2), R_f = 0.35. IR (KBr, cm^{-1}): 3424, 2960, 2865, 1630, 1464, 1383, 1351, 1106, 1062, 958, 761. **Aa-4** was identified as β-sitosterol by m.m.p, co-TLC, and superimposable IR spectrum with an authentic sample.

Triacontanoic acid (Aa-5)

Aa-5 was obtained as colourless solid from chloroform/methanol, yield 24 mg (0.002%), m.p. 80–81°C. TLC: $CHCl_3$/MeOH (9.4 : 0.6), R_f = 0.42. IR (KBr, cm^{-1}): 2931, 2843, 1695, 1475, 1463, 1350, 1180, 730, 720. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (3H, t, J = 6.5 Hz, CH_3), 1.60 and 1.25 (54 H, br s, 27 × —CH_2), 2.34 (2H, t, J = 7.5 Hz, —CH_2—CO—OH). EI-MS (70 eV) m/z (%): 452 (6), 424 (12), 396 (10), 368 (6), 340 (2). These spectral data are in close agreement with those reported for triacontanoic acid in the literature[15].

Kaempferol (Aa-6)

Aa-6 was obtained as yellow solid from hexane/EtOAc, yield 32 mg, (0.003%), m.p. 274°C. The compound gave positive test for flavonoid with Shinoda's reagent. TLC: $CHCl_3$/MeOH (19 : 1), R_f = 0.4. UV λ_{max} MeOH (nm): 264, 321 sh, 362; +NaOH 381 dec; + $AlCl_3$ 270, 335, 416; +$AlCl_3$ + HCl 276, 302sh, 355, 414; +NaOAc 273, 325, 379; +H_3BO_3 262, 367. IR (KBr, cm^{-1}): 3326, 3195, 1657, 1616, 1518, 1379, 1 170, 815, 784 and 637. ^{1}H NMR (400 MHz, acetone-d_6): δ 6.28 (1H, d, J = 2.1 Hz, H-6), 6.55 (1H, d, J = 2.1 Hz, H-8), 6.70 (1H, s, 3-OH), 7.15 (2H, d, H-3′ and H-5′), 8.15 (2H, d, H-2′ and H-6′), 9.31 (1H, s, 7-OH), 10.10 (1H, s, 4′-OH) and 12.20 (1H, s, 5-OH). ^{13}C NMR (100 MHz, acetone-d_6): ppm 146.3 (C-2), 136.5 (C-3), 176.4 (C-4), 162.5 (C-5), 99.3 (C-6), 165.3 (C-7), 94.6 (C-8), 157.9 (C-9), 104.2 (C-10), 123.4 (C-1′), 130.6 (C-2′ and C-6′), 116.5 (C-3′ and C-5′), 160.4 (C-4′). EI-MS (70 eV): 287, 286, 258, 242, 216, 184, 153, 147, 121, 105. On the basis of the above data and by comparison with literature data[16, 17] **Aa-6** has been identified as 3,5,7,4′-tetrahydroxyflavone/ kaempferol. The identity was further confirmed by m.m.p, co-TLC and superimposable IR spectrum with an authentic sample.

Quercetin (Aa-7)

Aa-7 was obtained as yellow crystals from $CHCl_3$/EtOAc, yield. 32 mg (0.003%), m.p. 315°C. It gave positive test for flavonoid with Shinoda's reagent and positive test for phenols with neutral ferric chloride. TLC: $CHCl_3$/MeOH (9 : 1), R_f = 0.8. UV λ_{max} MeOH (nm): 262, 303 sh, 372; +NaOH 327 dec.; +$AlCl_3$

275, 338, 460; +$AlCl_3$ + HCl 267, 303 sh, 362, 431; +NaOAc 270, 326, 392; +H_3BO_3 261, 387. IR (KBr, cm^{-1}): 3403, 3318, 1630, 1611, 1310, 1170, 1016, 815, 714. ^1H NMR (400 MHz, acetone-d_6): δ 12.20 (1H, s, 5-OH), 10.09 (1H, s, 4′-OH), 8.83 (1H, s, 7-OH), 8.5 (1H, s, 3′-OH), 8.14 (1H, s, 3-OH), 7.82 (1H, d, J = 2.0 Hz, H-2′), 7.69 (1H, dd, J = 2.0 Hz and 8.5 Hz, H-6′), 7.0 (1H, d, J = 8.5 Hz, H-5′), 6.52 (1H, d, J = 1.2 Hz, H-8), 6.27 (1H, d, J = 1.3 Hz, H-6). ^{13}C NMR (100 MHz, acetone-d_6): ppm 146.9 (C-2), 136.7 (C-3), 176.5 (C-4), 162.2 (C-5), 99.1 (C-6), 164.9 (C-7), 94.4 (C-8), 157.7 (C-9), 104.0 (C-10), 123.6 (C-1′), 115.6 (C-2′), 145.9 (C-3′), 148.4 (C-4′), 116.1 (C-5′), 121.4 (C-6′). EI-MS (70 eV) m/z (%): 303 (4), 302 (20), 301 (24), 271 (12), 203 (10), 198 (18), 170(16), 158(14), 153 (10), 137 (12), 128 (15), 109 (8). The above data are in agreement with those reported for quercetin in literature[16, 17] and hence compound **Aa-7** has been identified as 3,5,7,3′,4′-pentahydroxy-6-methoxyflavone (quercetin).

Vanillic acid (Aa-8)

Colourless crystalline solid from chloroform/methanol, yield 25 mg, (0.0025%), m.p. 210°C. TLC: $CHCl_3$/MeOH (8.5 : 1.5), R_f = 0.4. IR (KBr, cm^{-1}): 3482, 1630, 1598, 1421, 1349, 881, 636. ^1H NMR (400 MHz, acetone-d_6): δ 3.88 (3H, s, C_3-OMe), 6.89 (1H, d, J = 8.32, H-5), 7.54 (1H, d, J = 1.96, H-2), 7.56 (1H, dd, J = 8.32 and 1.96 Hz, H-6). ^{13}C NMR (100 MHz, acetone-d_6): ppm 122.8 (C-1), 115.7 (C-2), 152.0 (C-3), 148.0 (C-4), 113.4 (C-5), 124.7 (C-6), 167.6 (—COOH), 56.2 (—OCH_3). EI-MS (70 eV) m/z (%): 168 (60), 153 (36), 138 (28), 125 (24), 107 (12), 91 (100) These data suggest that **Aa-8** is a phenolic acid. Comparison of the above spectral data with those reported in literature[18] confirmed the identity of **Aa-8** as 3-methoxy-4-hydroxy benzoic acid (vanillic acid).

Quercetrin (Aa-9)

Aa-9 was obtained as yellow crystals from chloroform, yield 42 mg (0.004%), m.p. 189°C. The compound gave positive Shinoda's and Molisch's tests suggesting that it is a flavonoid glycoside. TLC: $CHCl_3$/MeOH (8 : 2), R_f = 0.51. UV λ_{max} MeOH (nm): 256, 352; +NaOH 270, 331, 397; +$AlCl_3$ 270, 303 sh, 335, 432; +$AlCl_3$ + HCl 276, 302, 345, 397; +NaOAc 270, 325 sh, 379; +NaOAc + H_3BO_3 261, 367. IR (KBr, cm^{-1}): 3495, 3405, 2965, 2741, 1693, 1630, 1584, 1423, 1383, 1044, 866, 778, 722. ^1H-NMR (300 MHz, methanol-d_4): δ 7.29 (1H, d, J = 2.1 Hz, H-2′), 7.33 (1H, m, J = 1.9 and 8.1 Hz, H-6′), 6.90 (1H, d, J = 8.1 Hz, H-5′), 6.37 (1H, d, J = 2.04 Hz, H-8), 6.20 (1H, d, J = 2.04 Hz, H-6), 5.34 (1H, d, J = 1.6 Hz, H-1″), 4.21 (1H, m, H-2″), 3.74 (1H, dd, J = 3.3 and 9.2 Hz, H-3″), 3.35 to 3.41 (unresolved rhamnose protons), 0.93 (3H, d, J = 5.9 Hz, H-6″). ^{13}C NMR (75 MHz, methanol-d_4): ppm 159.3 (C-2), 136.2 (C-3), 179.6 (C-4), 163.2 (C-5), 99.7 (C-6), 165.8 (C-7), 94.6 (C-8), 158.5 (C-9), 103.5 (C-10), 122.8 (C-1′), 116.3 (C-2′), 146.4 (C-3′), 149.8 (C-4′), 116.9 (C-5′), 122.9 (C-6′), 103.5 (C-1″), 71.9 (C-2″), 72.1 (C-3″), 72.0 (C-4″), 73.2 (C-5″), 17.6 (C-6″). EI-MS (70 eV) m/z: 302 (base peak), 273, 245, 218, 189, 163, 153, 129, 128, 111. Based on all these evidences and literature data[16, 17] **Aa-9** has been identified as quercetin 3-O-β-D-rhamnoside (quercetin).

Kaempferol 4′-O-β-D glucopyranoside (Aa-10)

Aa-10 was obtained as yellow crystals from methanol, yield 10 mg (0.001 %), m.p. 289°C. It gave positive tests for flavonoids and carbohydrates with Shinoda's reagent and Molisch's reagent indicating that it is a flavonoid glycoside. TLC: chloroform/methanol (7.5 : 2.5), $R_f = 0.60$. UV λ_{max} MeOH (nm): 255 sh, 266, 323, 366; +NaOH 285, 323 sh, 412; +$AlCl_3$ 267 sh, 301, 353 sh, 425; +$AlCl_3$ + HCl 266 sh, 300, 352, 424; +NaOAc 275, 307 sh, 384; + H_3BO_3 268, 316, 367. IR (KBr, cm^{-1}): 3855, 3752, 3650, 3386, 1630, 1598, 1509, 1458, 1383, 1305, 1272. ^{1}H-NMR (300 MHz, DMSO-d_6/MeOH-d_4): δ 12.21 (1H, s, 5-OH), 9.95 (1H, s, 7-OH), 9.40 (1H, s, 3-OH), 7.32 (2H, d, J = 8.5 Hz, H-2′ and H-6′), 6.53 (2H, d, J = 8.6 Hz, H-3′ and H-5′), 5.96 (1H, d, H-8), 5.83 (1H, d, H-6), 5.05 (1H, anomeric proton of glucose), 3.25–4.14 (unresolved multiplet, glucose protons). EI-MS (70 eV): 448, 440, 358, 287 (base peak), 259, 230, 184, 153, 128, 121. Hydrolysis of the compound (3 mg) with 2 N HCl yielded glucose and kaempferol suggesting a O-glycosidic structure[19]. The identity of the aglycon as kaempferol was confirmed by co-TLC and superimposable IR spectrum with an authentic sample. The sugar moiety was identified as glucose by co-paper chromatography. Based on these experimental and literature evidence[20] **Aa-10** has been identified as kaempferol 4′-*O*-β-D-glucopyranoside.

TABLE-1
IN VITRO ANTIBACTERIAL ACTIVITIES OF THE EXTRACTS FROM *A. ARNOTTIANUM*

MTCC No.	Bacteria	Diameter of zone of inhibition in mm including the diameter of the disc		
		Petroleum extract (100 µg/disc)	Methanol extract (100 µg/disc)	Streptomycin extract (2 µg/disc)
	Gram-positive			
441	*Bacillus subtilis*	7.0	6.0	20.0
740	*Staphylococcus aureus* subsp. *aureus*	7.0	—	16.0
2940	*Staphylococcus aureus subsp. aureus*	8.5	9.0	14.5
	Gram-negative			
97	*Serratia marcescens*	—	6.0	18.0
103	*Pseudomonas fluorescens*	8.5	10.5	20.0
109	*Klebsiella pneumoniae* subsp. *pneumoniae*	14.0	12.0	20.0
443	*Escherichia coli*	9.0	7.0	9.0
733	*Salmonella typhi*	7.0	—	25.0
741	*Pseudomonas aeruginosa*	16.0	12.0	18.0
2682	*Arthrobacter protophormiae*	7.5	6.0	11.0

Experiments were done in triplicate and results are mean values. —, No inhibition.

The petroleum ether and methanol extracts of the dried whole plant *A.arnottianum* exhibited moderate inhibitory activity against both gram-positive and gram-negative bacteria. The petroleum ether extract of the plant was moder-

ately active against most of the tested gram-positive and gram-negative bacteria *Bacillus subtilis, Staphylococcus aureus* subsp. *aureus, Arthrobacter protophormiae, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*, while the MeOH extract showed only mild inhibition against few tested bacteria. However, the MeOH extract was more active than the petroleum ether extract against the bacteria *Staphylococcus aureus* subsp. *aureus* and *Pseudomonas fluorescens*. Both the extracts are significally active against the bacteria *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

TABLE-2
IN VITRO ANTIMICROBIAL STUDY OF THE COMPOUNDS ISOLATED FROM *A. ARNOTTIANUM*

MTCC No.	Bacteria	Diameter of zone of inhibition in mm including the diameter of disc								
		Aa-1	Aa-2	Aa-3	Aa-5	Aa-6	Aa-7	Aa-8	Aa-9	ST
	Gram-positive									
441	*Bacillus subtilis*	7	–	*	7	7	8	7	–	20
740	*Staphylococcus aureus* subsp. *aureus*	7	6	–	6	–	–	–	–	16
2940	*Staphylococcus aureus* subsp. *aureus*	6	–	–	–	9	8	9	–	20
2682	*Arthrobacter protophormiae*	–	–	*	–	–	–	7	–	10
	Gram-negative									
97	*Serratia marcescens*	–	7	6	7	–	6	–	–	18
103	*Pseudomonas fluorescens*	–	7	7	–	–	6	–	–	18
109	*Klebsiella pneumoniae* subsp. *pneumoniae*	6	*	*	–	9	7	7	–	14
426	*Proteus vulgaris*	–	*	6	–	–	8	7	–	16
443	*Escherichia coli*	8	*	*	6	9	7	8	6	11
733	*Salmonella typhi*	–	7	–	–	–	8	–	–	21
741	*Pseudomonas aeruginosa*	–	*	*	–	–	–	–	–	9

Experiments were done in triplicate and results are mean values.
ST, Streptomycin; reference (2 µg/disc); –, No inhibition, *, not done,

Aa-1 = lupeol acetate, **Aa-2** = lupeol, **Aa-3** = dotriacontanol, **Aa-5** = triacontanoic acid, **A-6** = kaempferol, **Aa-7** = quercetin, **Aa-8** = vanillic acid, **Aa-9** = quercetrin. (each 100 µg/disc).

A perusal of Table-2 reveals that except for compound **Aa-3** (dotriacontanol) all the other compounds are moderately active against the bacteria *Bacillus subtilis* and *Escherichia coli* and this complements the antibacterial activity of petroleum ether and methanol extracts against these bacteria. None of the compounds examined is active against the tested bacteria *Pseudomonas aeruginosa*. The activity of the petroleum ether extract agaist the bacteria *Staphylococcus aureus* subsp. *aureus* is mainly due to the compounds **Aa-2** (lupeol) and **Aa-5** (triacontanoic acid). Compound **Aa-1** (lupeol acetate) is active against five of the tested bacteria while compounds **Aa-2** (lupeol), **Aa-5** (lupeol acetate) and **Aa-6** (kaempferol) are active against four of the tested bacteria. Of the compounds isolated from methanol

extract, **Aa-7** (quercetin) is active against most of the tested bacteria. **Aa-8** (vanillic acid) is active against six bacteria while **Aa-9** (quercetrin) is active against only one of the tested bacteria.

Thus the study has revealed for the first time that petroleum ether and methanol extracts of *A. arnottianum* are moderately active against certain gram-positive and gram-negative bacteria. Among the constituents isolated from the petroleum ether extract of *A. arnottianum* lupeol acetate, lupeol and triacontanoic acid showed moderate activity. Quercetin and vanillic acid isolated from the MeOH extract of the plant showed moderate activity against gram-positive and gram-negative bacteria.

The antioxidant effects of compounds isolated from the plant *A. arnottianum* are reported in Table-3.

TABLE-3
EFFECTS OF COMPOUNDS ISOLATED FROM THE PLANT *A. ARNOTTIANUM*, ON MDA PRODUCTION IN RAT LIVER HOMOGENATE INDUCED BY $FeCl_2$-ASCORBIC ACID *IN VITRO**

Compounds (15 µg/mL)	Absorbance	MDA (nmol/g liver tissue wet wt.)	Inhibition (%)
Normal control* (L.H. + tris buffer)*	0.174	2.2	–
Induced control (L.H. + tris buffer + Fe^{2+} + AA)	0.549	7.0	–
Aa-1 (L.H. + tris buffer + Fe^{2+} + AA+TC	0.434	5.5	21.1
Aa-3 (L.H. + tris buffer + Fe^{2+} + AA+TC	0.434	5.5	21.1
Aa-5 (L.H. + tris buffer + Fe^{2+} + AA+TC	0.406	5.1	39.5
Aa-6 (L.H. + tris buffer + Fe^{2+} + AA+TC	0.215	2.7	89.5
Aa-7 (L.H. + tris buffer + Fe^{2+} + AA+TC	0.190	2.4	95.8
Aa-8 (L.H. + tris buffer + Fe^{2+} + AA+TC	0.662	8.4	–29.1
Aa-9 (L.H. + tris buffer + Fe^{2+} + AA+TC	0.221	2.8	87.5
Aa-10 (L.H. + tris buffer + Fe^{2+} + AA+TC	0.231	2.9	85.4

Experiments were done in duplicate and results are mean values.
*L.H., Liver homogenate; AA, Ascorbic acid; T.C., Test compound.

Aa-1 = lupeol acetate, **Aa-3** = dotriacontanol, **Aa-5** = triacontanoicacid, **Aa-6** = kaempferol, **Aa-7** = quercetin, **Aa-8** = vanillic acid, **Aa-9** = quercetrin and **Aa-10** = kaempferol 4′-O-β-D glucopyranoside.

The results showed that flavonoids have more antioxidant activity than the other compounds studied. All the flavonoids **Aa-6** (kaempferol) and **Aa-7** (quercetin) and the flavonoid glycosides **Aa-9** (quercetrin) and **Aa-10** (kaempferol 4′-O-β-D-glucopyranoside) showed more than 80% antioxidant activity at concentration of 15 µg/mL, while **Aa-8** (vanillic acid) induces oxidation at this concentration. Compounds **Aa-1**, **Aa-3** and **Aa-5** did not show any significant antioxidant activity. Out of several flavonoids tested for their antioxidant activity quercetin has been found to show maximum activity probably because of the 3,5,7,3′,4′-pentahydroxy substitution, particularly the 3′,4′-catechol grouping on the B ring and free OH group at position 3 of the flavonoid nucleus. Glycosilation of the 3-OH group reduces the free radical scavenging activity as observed in quercetin (**Aa-9**). The

same observation is also shown by compounds kaempferol (**Aa-6**) and kaempferol glycoside (**Aa-10**). Kaempferol has more antioxidant activity than kaempferol glycoside. In the case of quercetin the presence of catechol moiety in the B ring (3′,4′-OH) increases the antioxidant activity, while in kaempferol the less antioxidant activity is due to the absence of catechol moiety. Thus the study confirms that for flavonoids, to act as antioxidants, the presence of free hydroxyl groups and catechol moiety in ring B of the flavonoid nucleus are essential requirements. An understanding of the structural features, thus, will be of great help in predicting the biological activity of natural products.

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