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Phytochemical constituents of *Actiniopteris radiata* Linn. whole plant

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Abstract

Three steroidal compounds (β-sitosterol, β-sitosterol palmitate, β-sitosterol-D-glucoside), two alkane hydrocarbon chains (hentriacontane, hentriacontanol) and a flavonoid glycoside (quercetin-3-rutinoside) have been isolated from whole plant of *Actiniopteris radiata* Linn. and their structures were established by spectral analysis and direct comparison with authentic samples. This is the first report of occurrence of these compounds from *Actiniopteris radiata.*

Key words: *Actiniopteris radiata,* steroids, flavonoids, alkane hydrocarbons, Actiniopteridaceae.

Introduction

Actiniopteris radiata Linn. is a tiny terrestrial fern, found throughout the India and also in Burma, Srilanka, Afghanistan, Persia, Arabia, South Eastern Egypt. It belongs to the family Actiniopteridaceae. The term Actiniopteris originates from Greek word aktis (ray) and pteris (fern) which refers to "radiating leaf segments" (Mrittunjai *et al*., 2005). Its vernacular names are : mayurishika (in Sanskrit), nemali adugu (in Telugu). The plants are 8-25 cm height, rooting in crevices of rocks or between the joints of brick wall in moist and shady places. The rhizome is oblique to horizontal, 1.5-2.0 cm in length, densely covered with wiry roots. The young leaves show circinate vernation but the lamina becomes flat at an early stage of development. The lamina is stiff and rough to touch. The paste of leaves is used as anthelmintic, antimicrobial (Pradeep *et al*.,2010), antibacterial, antifungal (Naik and Jadge 2010), analgesic, (Jadge and Naik 2010), antifertility and also in tuberculosis. Similarly, the paste of leaves mixed with fresh cow milk is taken for irregularity in menstrual period. Major phytoconstituents present in stem and leaves are hentriacontane, hentriacontanol, β-sitosterol, β-sitosterol-D-glucoside and β-sitosterol palmitate. But as per our knowledge, there is no literature for isolation and characterization of above mentioned compounds from *Actiniopteris radiata.* Hence, the present research work was focused on the isolation and characterization of major sterols, alkane hydrocarbons and flavonoid, from the whole plant of *Actiniopteris radiata.*

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Materials and Methods

Collection and identification

The whole plant of *Actiniopteris radiata* Linn. was collected from Golkonda Fort, Hyderabad. It was authenticated by Professor B. Bhadraiah, Head, Department of Botany, Osmania University, Hyderabad, A.P., India.

Extraction and isolation

The whole plants of *Actiniopteris radiata* were extracted (3.5 kg) successively with petroleum ether (60- 80º), ethyl acetate and ethanol, respectively. These extracts were concentrated to remove the solvents. The yields of petroleum ether, ethyl acetate and ethanolic extracts were found to be 30g, 23g, 33g, respectively.

Petroleum ether extract

The concentrated petroleum ether extract (30 g) was dissolved in petroleum ether (20 ml) and chromatographed through a column of silica gel 60-120 mesh LR (diam. 4 cm × length 45 cm). The column being successively eluted with increasing polarities like petroleum ether, petroleum ether: benzene,

benzene, chloroform. The elution was carried out with petroleum ether: benzene in graded mixture *i.e.*, 95:05, 90:10, 85:15, 80:20,……up to 10:90. And finally carried with 100% benzene. Next elution was carried out with benzene: chloroform in a graded mixture (95:05, 90:10, 85:15….up to 40:60). From above elution, four different fractions were collected (*i.e.,* fraction A, B, C & D).

Fraction A, eluted from petroleum ether: benzene (95:05), resulted in a single compound which was confirmed by TLC (petroleum ether: benzene, 9:1). On evaporation, it gave a waxy substance. It was recrystalized with acetone. The product was designated as compound AR_1.

***Compound* AR_1**

IR (KBr cm^{-1}, λ max) : 2920 cm^{-1}, 2850 cm^{-1}, 1465 cm^{-1}, 1380 cm^{-1}, 725 cm^{-1} and 714 cm^{-1}.

^{1}HNMR δ values ppm: 1.31 (m, 4H, C-16, C-30), 1.29 (m, 8H, adjacent free methyl groups, C-14, 15, 28, 29), 1.26 (m, 42H, methylene groups), 0.88 (m, 8H, free methyl portion).

^{13}CNMR ($CDCl_3$): δ 31.9 (C-15, 29), 29.6 (C-1to13 and C-18 to27), 29.3 (C-14, 28), 22.7 (C-16, 30), 14.1 (C-17, 31).

Mass Spectrum (MS) showed M^+ at m/z 436, corresponding to the molecular formula ($C_{31}H_{64}$). The maximum peak was that of the ion M-14 due to splitting of CH_2 groups.

It was found to be identical with **hentriacontane** (Figure 1) in comparision with authentic sample (mixed m.p. CO-TLC and superimpossible IR).

Fraction B, eluted from petroleum ether: C_6H_6 (85:15), gives mixture of steroidal compounds. It was further subjected to rechromatography with petroleum ether: C_6H_6 (85: 15, 83: 17, 80: 20, 75: 25, and 70: 30). The elution was carried with 80: 20, gave single compound. It was designated as compound AR_2.

***Compound* AR_2**

IR (KBr, γ max): 3374 cm^{-1}, 2944 cm^{-1}, 2871 cm^{-1}, 1641 cm^{-1} , 1381 cm^{-1}, 1095 cm^{-1} , 996 cm^{-1}.

^{1}HNMR δ values ppm : 5.37 (m , 1H, vinyl portion, C-14), 3.99 (s,1H, c-3), 2.35 (m, 2H, adjacent to carboxylic group), 2.33-2.04 (m, 3H, C-12, C-15), 1.8-1.66 (m, 3H, C- 2,15,27), 1.64-1.60 (m, 5H, C-10,11,23,26,30), 1.56-1.52 (m, 2H, C-16,17), 1.47-1.44 (m, 3H, C-5,6,9), 1.41-35 (m, 5H, C-2,3,7,10,11), 1.31-1.30 (m, 2H, C-17,21), 1.29 (m, 2H, C-32,37), 1.27 (m, 1H, C-16), 1.26 (m, 5H, C-32,36), 1.25 (m, 2H, C-24,25), 1.13 (s, 1H, C-3), 1.04 (s, 1H, C-22), 0.96 (m, 2H, C-44,46), 0.91 (m, 2H, C-28,47).

MS showed M^+ at m/z 622, corresponding to the molecular formula ($C_{45}H_{79}O_2$). The other characteristic peaks were at m/z 208 [m-side chain at ring A]$^+$, 141 [m-side chain at ring D].

It was found to be identical with **β-sitosterol palmitate** (Figure 2) in comparison with authentic sample (mixed m.p. CO-TLC and super impossible IR).

Fraction C, eluted from pure benzene (100%) on evaporation gave single compound, recrystalized with acetone. It was designated as compound AR_3.

***Compound* AR_3**

IR (KBr, γmax) : 3600 cm^{-1}, 2940 cm^{-1}, 2920 cm^{-1}, 2860 cm^{-1} , 1480 cm^{-1}, 1390 cm^{-1}.

^{1}HNMR δ values ppm : 3.65 (s, 1H, -OH group), 3.50 (t, 2H, adjacent to CH_2OH), 1.53 (m, 2H, C-16), 1.43 (m, 2H, C-15), 1.31-1.26 (m, 50H, methylene groups), 0.88 (m, 3H, free methyl portion).

^{13}CNMR ($CDCl_3$): 62.8 (C-17), 32.2 (C-16), 31.9 (C-29), 29.6 (C-1to13 and C-18 to27), 29.3 (C-28), 22.7 (C-30), 14.1 (C-31).

MS showed M^+ at m/z 452, corresponding to the molecular formula ($C_{31}H_{64}O$). The maximum peak was that of the ion M-14 due to splitting off of CH_2 groups.

It was found to be identical with **hentriacontanol** (Figure 3) in comparison with authentic sample (mixed m.p. CO-TLC and super impossible IR).

Fraction D, eluted from benzene: $CHCl_3$ (50:50), gave single spot on TLC (C_6H_6: $CHCl_3$, 40:60). It was designated as compound AR_4.

***Compound* AR_4**

IR (KBr, γ max): 3374 cm^{-1}, 2944 cm^{-1}, 2871 cm^{-1}, 1641cm^{-1}, 1381 cm^{-1}, 1095 cm^{-1} , 996 cm^{-1}.

^{1}HNMR δ values ppm: 5.2 (m, 1H, vinyl proton, H-6), 3.5 (s, 8H, CH-0H, H-3), 2.1 to 1.49 (m, 8H, methane propions), 1.38 to 1.2 (m, 22H, $11CH_2$), 1.1 to 0.9 (m, 18H, methyl portion).

^{13}CNMR ($CDCl_3$): δ 31.0 (C-1), 30.4 (C-2), 37.2(C-3), 46.9 (C-4), 139.6 (C-5), 121.0 (C-6), 31.3 (C-7), 31.8 (C-8), 50.5 (C-9), 36.4 (C-10), 21.3 (C-11), 40.0 (C-12), 42.2 (C-13), 55.3 (C-14), 23.7 (C-15), 28.7 (C-16), 55.3 (C-17), 13.9 (C-18), 19.3 (C-19), 35.6 (C-20), 18.4 (C-21), 34.8 (C-22), 30.0 (C-23), 41.5 (C-24), 33.8 (C-25), 21.5 (C- 26), 22.5 (C-27), 106.0 (C-28), 28.1 (C-29).

MS showed m+ at m/z 414, corresponding to the molecular formula ($C_{29}H_{50}O$).

It was found to be identical with **β-sitosterol** (Figure 4) in comparison with authentical sample (mixed m.p. CO-TLC and super impossible IR).

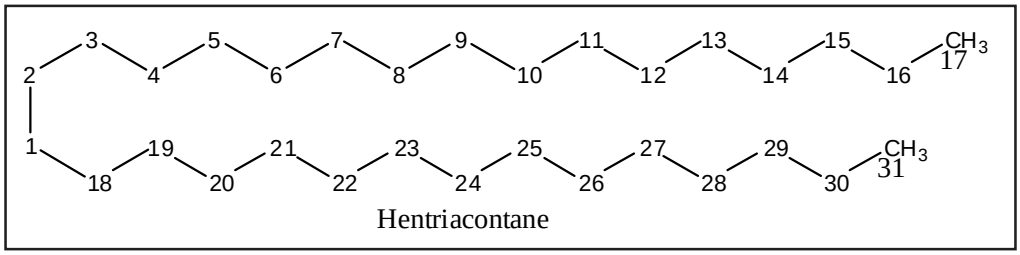


Figure 1

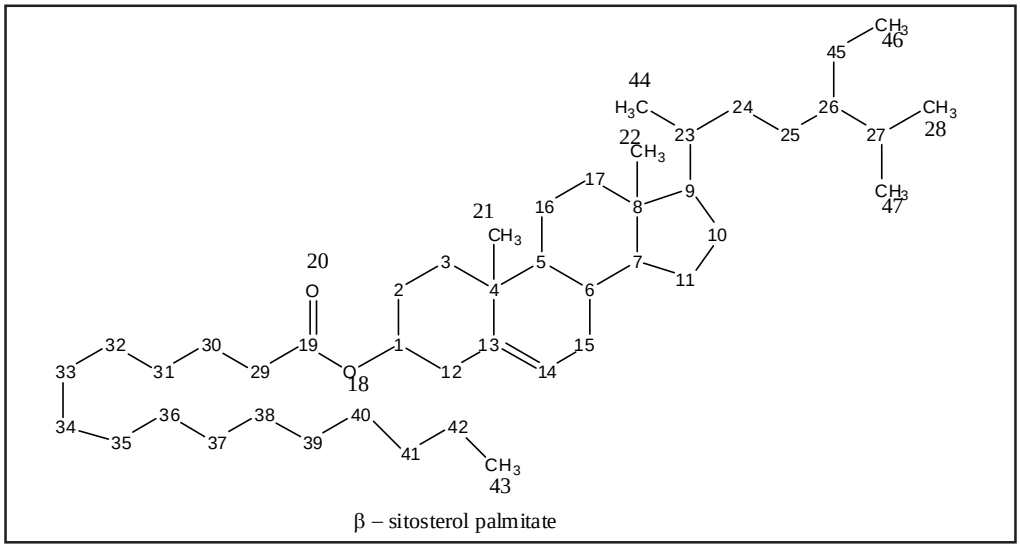


Figure 2

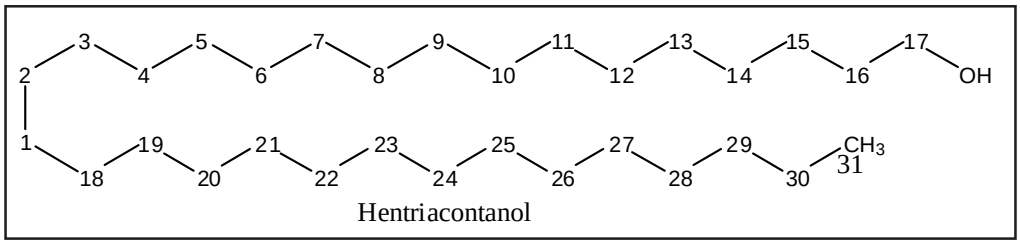


Figure 3

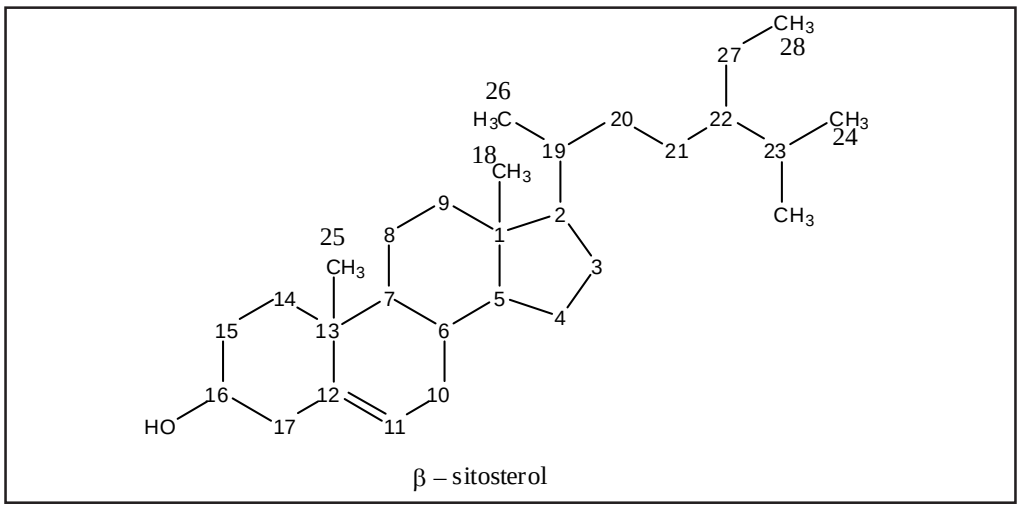
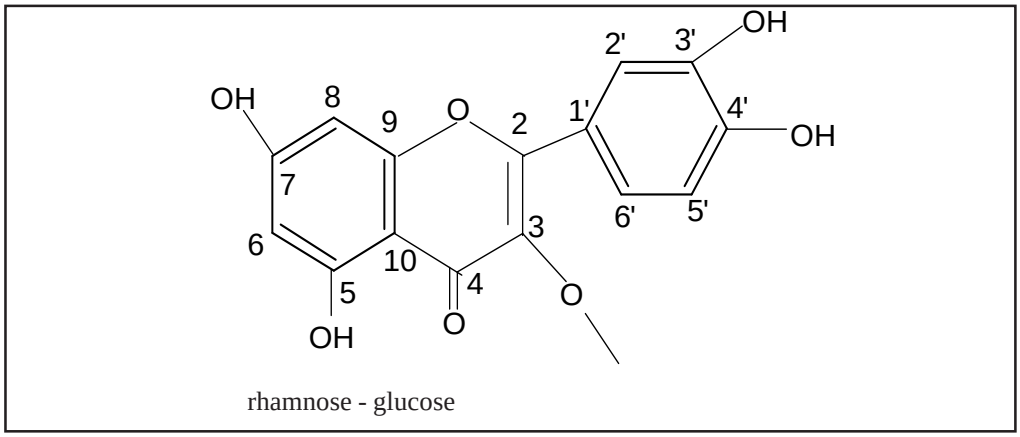
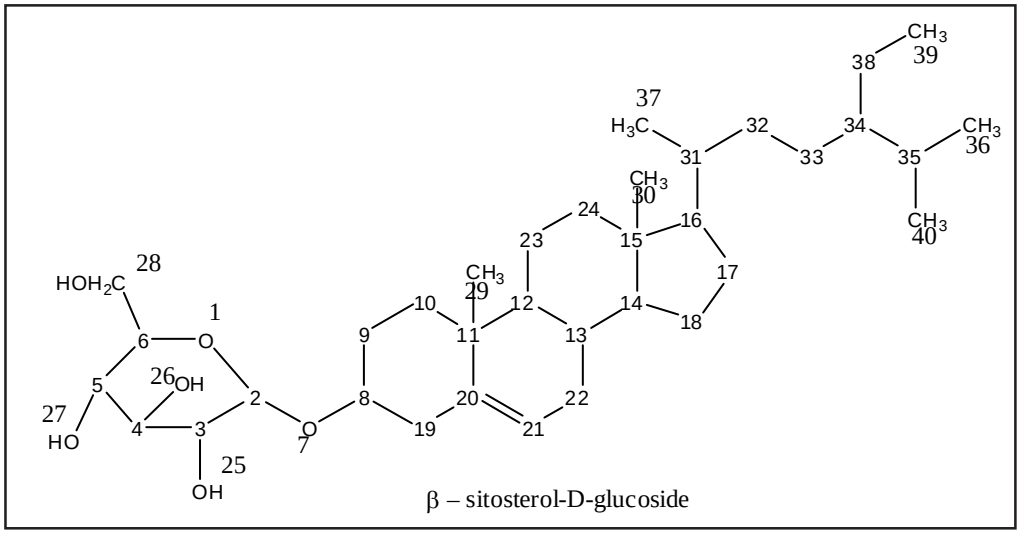


Figure 4



rhamnose - glucose

Figure 5



β – sitosterol-D-glucoside

Figure 6

Ethyl acetate extract

The concentrated ethyl acetate extract (23 g) was dissolved in ethyl acetate (20 ml) and chromatographed through a column of Silica gel 60-120 mesh LR (diam. 4 cm × length 45 cm). The column being successively eluted with ethyl acetate: chloroform. The elution was carried out with ethyl acetate: chloroform in graded mixture *i.e.*, 95:05, 90:10, 85:15, 80:20…up to 50:50). At 90: 10 (ethyl acetate: chloroform), a single compound was eluted, which was confirmed by TLC (Toluene: MeOH, 4:1) and designated as compound AR_5.

***Compound* AR_5**

IR(KBr cm^{-1}): 3421.4 cm^{-1}, 2954.8 cm^{-1}, 1658.5 cm^{-1}, 1598.3 cm^{-1}, 1469.6 cm^{-1}, 1206.2 cm^{-1}.

^{1}**H**NMR $(DMSO\text{-}d)_6$: 12.58 (s, 1H, 5 0H), 10.89 (s, 1H, 3 0H), 9.6 (s, 1H, 7 0H), 9.1(1H, 4'-OH), 9.2 (1H, 5'-OH), 6.8 (d, 1H, 1H, H-8), 6.37 (d, 1H, H-7), 6.17-6.18 (d, 1H, H 5',6'), 7.5 (d, 1H, H-2'), 5.05-5.44 (m, 1H, H-1,3,4 glucosyl; m, 1H, H-1,2,3,4 rhamnosyl), 3.2-3.9 (m, rhamnoglycosyl), 1.13 (d, 3H rhamnosyl CH_3).

^{13}CNMR ($CDCl_3$): 156.37 (C-2), 133.26 (C-3), 177.327 (C-4), 161.18 (C-5), 98.61 (C-6), 164.00 (C-7), 93.52 (C-8), 156.55 (C-9), 103.92 (C-10), 121.53 (C-1'), 115.17 (C-2'), 144.69 (C-3'), 148.36 (C-4'), 116.22 (C-5'), 121.13 (C-6'), 101.14 (C-1''), 74.03(C-2''), 75.87 (C-3''), 69.96 (C-4''), 76.41 (C-5''), 66.643 (C-6''), 100.69 (C-1'''), 70.32 (C-2'''), 70.52 (C-3'''), 71.80 (C-4'''), 68.18 (C-5'''), 17.67 (C-6''').

MS showed M^+ at m/z 610, corresponding to the molecular formula ($C_{27}H_{30}O_{16}$).

It was found to be identical with **quercetin-3-rutinoside** on comparison with authentic sample (mixed m.p. CO-TLC and superimpossible IR).

Ethanolic extract

The concentrated ethanolic extract (33g) was dissolved in chloroform (20 ml) and chromatographed through a column of Silica gel 60-120 mesh LR (diam. 4 cm × length 45 cm). The elution was carried out with chloroform: methanol in graded mixture *i.e.*, 95:05, 90:10, 85:15, 80:20,…up to 50:50). At 10:1 (chloroform: methanol), a single compound was eluted, which was confirmed by TLC ($CHCl_3$: MeOH). It was designated as compound AR_6.

***Compound* AR_6**

IR(KBr cm^{-1}): 3650 cm^{-1}, 3400 cm^{-1}, 1275 cm^{-1}, 1225 cm^{-1}, 1050 cm^{-1}, 975 cm^{-1}, 900 cm^{-1}, 850 cm^{-1}.

^{1}HNMR δ values ppm : 5.37 (m, 1H, vinyl portion, C-21), 5.03 (d, 1H, C-2), 3.79 (m, 1H, C-28), 3.76(m, 1H, C-6), 3.73 (m, 1H, C-3), 3.65 (m, 1H, H-29), 5.38 (m, 3H, H-25,26,27), 3.54-3.40 (m, 3H, C-4,5,28), 2.87 (m, 1H, C-8), 2.21-2.04 (m, 2H, C-19,22), 1.96-1.79 (m, 2H, C-19,22), 1.60-1.62 (m, 4H, C-17,18,31,35), 1.56-1.31 (m, 11H, C-9,10,12,13,14,16,18,23,24), 1.30-1.27 (m, 5H, C-9,24,29), 1.25 (s, 6H, C-32,33,34), 1.09 (m, 3H, methyl portion, C-30), 0.96-0.91 (d, 9H, free methyl groups, C-36,37,38).

^{13}CNMR ($CDCl_3$): 140.8 (C-20), 121.8 (C-21), 109.9 (C-2), 81.9 (C-8), 81.5 (C-6), 76.8 (C-4), 74.1 (C-3), 71.5 (C-5), 62.2 (C-28), 56.5 (C-14), 56.2 (C-16), 50.8 (C-12), 42.7 (C-15), 39.9 (C-19,34), 39.8 (C-24), 37.7 (C-11), 37.5 (C-10), 36.1 (C-32), 35.8 (C-31), 32.0 (C-22), 31.8 (C-13), 29.8 (C-9), 28.1 (C-35), 26.3 (C-18), 25.9 (C-17), 24.6 (C-33), 23.2 (C-36,40), 21.1 (C-23), 19.4 (C-37), 19.3 (C-29), 12.0 (C-30).

MS showed m+ at m/z 576, corresponding to the molecular formula ($C_{35}H_{61}O_6$). The other characteristic peaks were at m/z 163 [m-sidechain at ring A]$^+$, 141 [m-side chain at ring D].

It was found to be identical with **β-sitosterol-D-glucoside** (Figure 6) on comparison with authentic sample (mixed m.p. CO-TLC and superimpossible IR).

Results and Discussion

Chromatographic resolution of petroleum ether, ethyl acetate and ethanol extracts of whole plant of *Actiniopteris radiata* Linn. furnished compound AR_1, AR_2, AR_3, AR_4, AR_5 and AR_6 which were characterized as **hentriacotane , β-sitosterol palmitate, hentriacotanol, β-sitosterol, quercitin-3-rutinoside and β-sitosterol-d-glucoside** (Figures 1-6)by detailed spectral analysis *i.e.*, I.R, ^{1}HNMR, ^{13}CNMR, MASS and direct comparison with authentic samples. All the above compounds are being reported for the first time from this plant.

Acknowledgement

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