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# PHYTOCHEMICAL INVESTIGATION OF THE FRONDS OF *ADIANTUM CAPILLUS-VENERIS* L.

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## ABSTRACT

*Adiantum capillus-veneris* L. (family Pteridaceae) is an aromatic, non-flowering fern with a creeping rhizome. Its fronds are used to treat chest diseases, headaches, insanity, jaundice and menstrual complaints. This research work was proposed to isolate chemical constituents isolated from the plant fronds and to characterize their structures. An ethanol extract of the fronds was fractionated with petroleum ether, ethyl acetate and methanol. The ethyl acetate fraction was adsorbed with silica gel for column, dried and chromatographed over a silica gel column packed in *n*-hexane. Various solvent mixtures of increasing polarity, viz., *n*-hexane, ethyl acetate and methanol were used to elute the column. The isolated chemical constituents were characterized as pentacyclic triterpenes (fern-9(11)-ene, **1**), 3-α-hydroxyfernane (farnan-3α-ol, **3**), farnan-3β, 16β-diol (**4**) and betulinic acid (**5**), an aromatic ester identified as 16′-methyl n-heptadecanyl 3,4-dihydroxybenzoate (**2**) and a rare diglucoside α-Dglucopyranosyl-(6→1′)-O- α-D-glucopyranoside (**6**). Their structures were established on the basis of spectral data analysis and chemical reactions.

**KEYWORDS:** *Adiantum capillus-veneris*, fronds, extraction, phytoconstituents, isolation, spectral data, characterization.

## INTRODUCTION

*Adiantum capillus-veneris* L., syn. *A. africanum* R. Br., *A. coriandrifolium* Lam., *A. fontanum* Salisb., *A. michelii* Christ, *A. paradiseae* Baker, *A. schaffneri* E. Fourn., *A. trifidum* Willd. ex Bolle (family Pteridaceae), known as hansraj, parsiya washan, maidenhair fern and venus hair fern, is a native to the southern United States, Mexico, South America, Eurasia, Western Asia and Australia; found in Afghanistan, China, India, Iran, Japan, Nepal and Sri Lanka. It is a hardy, aromatic, non-flowering plant with a creeping rhizome covered with narrow chaffy-brown scales, up to 45 cm high; plant fronds are double-rowed, tender, glabrous; petiole glossy black, shiny, slender, brown or black, coated with hair at the base; leaf blade oblong-ovate; leaflets lobed around the margins.[1] The plant is antirheumatic, antispasmodic, blood purifier, demulcent, diuretic, pectoral and tonic, used to treat boils, bronchitis, coughs, kidney stones, menstrual complaints, mental illness, rheumatism, strangury, tuberculosis, for detoxifying the liver and as a lotion for bumblebee and centipede stings.[2-4] The fronds are antidandruff, antitussive, astringent, demulcent, depurative, emetic, emmenagogue, emollient, expectorant, febrifuge, galactagogue, laxative, pectoral, refrigerant, stimulant, sudorific and tonic, beneficial to relieve bronchitis, chest diseases, cold, congestion, coughs, headaches, hoarseness, jaundice, menstrual complaints, as a detoxicant in alcoholism and to expel worms from the body; frond smoke is useful to cure insanity and respiratory tract disorders. [1-4] The leaves are abortifacient, antifungal and febrifuge, prescribed as a hair tonic and against bees stings, dandruff, infertility, menstrual complaints, snake bites and vaginal discharges.[1-4]

The fern fronds contained diacylglyceryl-*O*-4′-(*N*,*N*,*N*-trimethyl)-homoserine[5], pteron-14-en-7α-ol, fernene and adianene type- triterpenoids,[6] isoadiantone, isoadiantol-B, 3-methoxy-4-hydroxyfilicane, 3,4-dihydroxyfilicane, quercetin, quercetin-3-O-glucoside and rutin.[7], 30-normethyl fernen-22-one (capillirone), hopan-3β-ol (capillirol B), 4-α-hydroxyfilican-3-one and 3β,4α-dihydroxyfilicane.[8] The leaves yielded fernane, oleanane and hopene type-triterpenoids, isoadiantone, isoglaucanone, isoadiantol, hydroxyadiantone, filic-3-ene pteron-14-en-7a-ol, adian-5(10)-en-3a-ol, adian5-en-3a-

ol and 4-α-hydroxyfilican-3-one.[9-12] sulphate esters of hydroxycinnamic acid-sugar derivatives, rutin, quercetin, quercetin-3-O-glucoside, querciturone, isoquercitrin, nicotiflorin, naringin, astragalin, populnin, procyanidin, prodelphinidin, and kaempferol-3-sulfate.[13-16] a coumarin dimer, daphnoretin,[17] glycosides, phytosterols, oleanane triterpenes, ascorbic acid, imidazoles and carboxylic acids.[18] An essential oil of the aerial parts was composed of carvone (31.58%), carvacrol (13.75%), hexadecanoic acid, thymol, hexahydrofarnesyl acetone and n-nonanal.[19] The fern volatile components included (E)-2-decenal (32.1%), lauric amide (12.4%), (E)-2-heptenal, 2-phenylethanal, 2-phenylethanol, benzoic acid, 1-octen-3-ol, (E)-2-nonenal, (Z)-2-decenal, nonanoic acid, 2,4)-decadienal, octanoic amide, nonanoic amide, decanoic amide, undecanoic amide and myrcene.[20] The plant contained beta-sitosterol, stigmasterol and capesterol,[21] inden-4-yl and phenanthrene derivatives, myristic, palmitic and vaccenic acids, vitamin E and gamma-sitosterol.[22]

Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations the fronds of *Adiantum capillus-veneris* collected from North West Delhi were screened for the isolation and characterization of their chemical constituents.

## MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and analysis assays) were adopted from the earlier published work.[8]

### General procedures

The melting points were determined in one end open capillary tubes on a melting point M-560 apparatus (Perfit, India) heated thermoelectrically. UV spectra were determined with Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were recorded by using KBr pellets with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The $^{1}H$ (400 MHz) and $^{13}C$ (100 MHz) NMR spectra were recorded on Bruker DRX Spectrometer (Rheinstetten, 2 Germany) using $CDCl_3$ as a solvent and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass spectra were recorded on a Jeol JMS-D 300 instrument using Argon/Xenon gas as the FAB. *n*-Hexane, ethyl acetate, chloroform, methanol and other solvents of analytical grade were purchased from E. Merck (India) Ltd, New Delhi. Silica gel with 60-120 mesh particle size was procured from Qualigens, Mumbai, India) and used for column chromatography. The purity of the isolated compounds was checked on precoated TLC plates with Silica gel 60 $F_{254}$ (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapors or under UV radiations and spraying with ceric sulfate solution.

### Plant material

The fronds of *Adiantum capillus-veneris* were collected from Ashok Vihar Nursery, North West Delhi and authenticated by Dr. H. B. Singh, Taxonomist, National Institute of Science Communication and Information resources (NISCAIR), CSIR, New Delhi. A voucher specimen (No. 2009-10/1199/03) has been deposited in the herbarium of NISCAIR for future reference.

### Extraction and isolation

The fronds *A. capillus-veneris* were shade dried for three days, coarsely powdered (5.0 kg) and extracted with ethanol (95%) in a Soxhlet apparatus. The solvent was evaporated under reduced pressure to yield a dark brown viscous mass (11.4 % w/w). The extract was then fractionated with solvents of increasing polarity, viz. n-hexane, ethyl acetate and methanol to give 35%, 31.25% and 33.75% (w/w) products, respectively. A slurry of silica gel for column (60-120 mesh) was prepared by adsorbing the ethyl acetate extract (150 g) in a small amount of methanol. It was dried in air and chromatographed over a silica gel column (1.6 m x 16 mm x 2 mm) packed in *n*-hexane. Various solvent mixtures of increasing polarity, viz., *n*-hexane, *n*-hexane-ethyl acetate (9:1, 3:1, 1:1, 1:3, *v/v*), ethyl acetate and ethyl acetate - methanol (19.9: 0.1; 99: 1; 97: 3; 19: 1; 93: 7; 9: 1 , v/v) were used to elute the column. The fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same $R_f$ values were combined and crystallized. The isolated compounds were recrystallized to get the following pure compounds:

### Davallene (1)

Elution of the column with *n*-hexane - ethyl acetate (19:1) gave colourless flakes of **1,** recrystallized from ethyl acetate –methanol (1:1), yield 321 mg, $R_f$ : 0.8 (*n*-hexane-ethyl acetate, 99:1); m. p. 169 – 170 °C, $[\alpha]_D^{23}$ - 18.2 °; UV λmax (MeOH): 207 nm (log ε 5.3); IR $\upsilon_{max}$ (KBr): 2925, 2841, 1647, 1433, 1360, 1255, 1052 $cm^{-1}$; $^{1}H$ NMR ($CDCl_3$ ): δ 5.28 (1H, t, J = 2.4 Hz, H-11), 1.25 (3H, brs, Me-23), 1.05 (3H, brs, Me-25), 0.92 (3H, d, J = 5.4 Hz, Me-30), 0.88 (3H, d, J = 5.5 Hz, Me-29), 0.86 (3H, brs, Me-24), 0.84 (3H, brs, Me-27), 0.79 (3H, brs, Me-26), 0.75 (3H, brs, Me-28), 2.69-1.42 (25H, m, 10 x $CH_2$ , 5 x CH); $^{13}C$ NMR ($CDCl_3$ ): δ 42.41 (C-1), 36.18 (C-2), 41.48 (C-3), 37.96 (C-4), 44.84 (C-5), 18.02 (C-6), 19.76 (C-7), 49.34 (C-8), 151.66 (C-9), 37.67 (C -10), 115.60 (C-11), 42.95 (C-12), 38.04 (C-13), 40.56 (C-14), 28.25 (C-15), 36.76 (C-16), 42.76 (C-17), 52.01 (C-18), 19.52 (C-19), 29.75 (C-20), 59.65 (C-21), 39.96 (C-22), 30.82 (C-23), 14.01 (C-24), 25.08 (C-25), 23.05 (C-26), 22.15 (C-27), 15.84 (C-28), 15.45 (C-29), 21.70 (C-30); +ve FAB MS *m/z* (rel. int.): 410 $[M]^+$ ($C_{30}H_{50}$) (100).

### 16′-Methyl *n*-heptadecanyl protocatechuate (2)

Elution of the column with ethyl acetate afforded pale yellow crystals of **2**, recrystallized from acetone, yield 193 mg; m. p. 134 - 135 ° C; $R_f$ 0.71 (*n*-hexane - ethyl acetate, 7:3), UV λ max (MeOH): 205, 297 nm (log ε

5.3, 1.2); IR υmax (KBr): 3274, 2925, 2848, 1725, 1634, 1550, 1465, 1359, 1283, 1015, 727 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): : δ 6.51 (1H, d, J = 1.8 Hz, H-2), 6.48 (1H, dd, J = 1.8, 7.8 Hz, H-6), 6.01 (1H, d, J = 7.8 Hz, H-5), 4.01 (2H, t, J = 8.4 Hz, $H_2$-1′), 1.81 (1H, m, H-16′), 1.50 (4H, m, $H_2$-2′, $H_2$-3′), 1.31 (24H, brs, 12 x $CH_2$), 0.97 (3H, d, J = 7.2 Hz, Me-17′), 0.85 (3H, d, J = 6.9 Hz, Me-18′); $^{13}C$ NMR ( $CDCl_3$) : δ 145.82 (C-1), 122.05 (C-2), 167.29 (C-3), 150.14 (C-4), 116.60 (C-5), 115.29 (C-6), 173.83 (C-7), 67.31 (C-1′), 34.52 (C-2′), 31.56 (C-3′), 29.31 (C-4′), 29.24 (C-5′), 28.49 (C-6′), 28.31 (C-7′), 28.27 (C-8′), 28.24 (C-9′), 28.21 (C-10′), 28.21 (C-11′), 27.11 (C-12′), 26.23 (C-13′), 25.81 (C-14′), 22.61 (C-15′), 35.72 (C-16′), 14.82 (Me-17′), 14.63 (C-18′); +ve FAB MS *m/z* (rel. int.): 407 $[M+H]^+$ ($C_{25}H_{43}O_4$) (1.4), 363 (10.1), 269 (12.7), 253 (6.3), 153 (14.6), 137 (22.8).

**Farnan-3α-ol (3)**

Elution of the column with ethyl acetate – methanol (99:1) yielded colourless amorphous powder of **3**, recrystallized from acetone, m. p. 184 – 185 $^0$ C, $R_f$ 0.70 (*n*-hexane – ethyl acetate, 3 : 2); UV λmax 206 nm (log ε 4.9); IR $\upsilon_{max}$ (KBr): 3410, 2926, 2842, 1461, 1380, 1260, 1051, 881 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 3.73 (1H, dd, J = 5.3, 5.8 Hz, H-3β), 1.25 (3H, brs, Me-23), 1.17 (3H, d, J=6.3 Hz, Me-29), 0.99 (3H, d, J=7.5 Hz, Me-30), 0.97 (3H, brs, Me-27), 0.84 (3H, brs, Me-24), 0.81 (3H, brs, Me-26), 0.79 (3H, brs, Me-25), 0.68 (3H, brs, Me-28), 2.40 – 1.28 (26 H, m, 10 x $CH_2$, 6 x CH); $^{13}C$ NMR ($CDCl_3$): δ 39.70 (C-1), 32.68 (C-2), 72.84 (C-3), 32.83 (C-4), 48.53 (C-5), 18.70 (C-6), 23.85 (C-7), 47.45 (C-8), 50.41 (C-9), 37.40 (C-10), 32.78 (C-11), 40.31 (C-12), 41.91 (C-13), 14.97 (C-14), 24.29 (C-15), 33.25 (C-16), 45.06 (C-17), 54.98 (C-18), 20.90 (C-19), 29.70 (C-20), 56.11 (C-21), 31.93 (C-22), 33.41 (C-23), 14.13 (C-24), 21.60 (C-25), 21.75 (C-26), 16.72 (C-27), 15.86 (C-28), 15.21 (C-29), 16.75 (C-30); +ve FAB MS *m/z* (rel. int.): 429 $[M+H]^+$ ($C_{30}H_{53}O$) (8.8), 413 (12.6), 410 (100), 398 (58.4).

**Farnan-3β, 16β-diol (4)**

Elution of the column with ethyl acetate – methanol (49:1) yielded colourless amorphous powder of **4**, recrystallized from acetone, yield 65 mg, m. p. 219 – 220 $^0$ C, $R_f$ 0.51 (*n*-hexane – ethyl acetate, 3 : 2); UV λmax 206 nm (log ε 4.7); IR $\upsilon_{max}$ (KBr): 3415, 3383, 2918, 2851, 1458, 1362, 1073 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) : δ 3.72 (1H, dd, J = 5.2, 8.8 Hz, H-3α), 3.63 (1H, dd, J = 5.5, 8.6 Hz, H-16α), 1.25 (3H, brs, Me-23), 1.15 (3H, d, J = 6.3 Hz, Me-30), 0.96 (3H, d, J = 5.5 Hz, Me-29), 0.94 (3H, brs, Me-27), 0.84 (3H, brs, Me-24), 0.81 (3H, brs, Me-25), 0.79 (3H, brs, Me-26), 0.60 (3H, brs, Me-28), 2.37 – 1.31 (24 H, m, 9 x $CH_2$, 6 x CH); $^{13}C$ NMR ($CDCl_3$) : δ 39.70 (C-1), 32.69 (C-2), 72.84 (C-3), 31.92 (C-4), 48.43 (C-5), 18.70 (C-6), 23.85 (C-7), 47.45 (C-8), 50.42 (C-9), 37.33 (C-10), 22.04 (C-11), 40.31 (C-12), 41.91 (C-13), 14.97 (C-14), 24.29 (C-15), 74.01 (C-16), 45.06 (C-17), 54.98 (C-18), 20.90 (C-19), 29.70 (C-20), 56.11 (C-21), 31.93 (C-22), 33.25 (C-23), 14.13 (C-24), 21.59 (C-25), 22.78 (C-26), 16.49 (C-27), 15.86 (C-28), 15.20 (C-29), 16.75 (C-30); +ve FAB MS *m/z* (rel. int.): 445 $[M+H]^+$ ($C_{30}H_{53}O_2$) (1.4), 276 (16.2), 250 (11.3), 194 (21.6), 168 (13.4), 140 (43.8).

**Betulinic acid (5)**

Further elution of the column with ethyl acetate – methanol (49:1) gave colourless crystals of **5**, recrystallized from chloroform –methanol (1:1), 321 mg, $R_f$ : 0.50 (chloroform: methanol, 99:1); m. p. 315 - 317 °C, UV λmax (MeOH): 211, 257 nm (log ε 4.8, 1.2); IR $\upsilon_{max}$ (KBr): 3505, 3245, 2931, 2940, 2872, 1691, 1635, 1456, 1383, 1239, 1181, 1037, 881 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ ): δ 4.77 (1H, s, $H_2$ -29a), 4.72 (1H, s, $H_2$ -29b), 3.80 (1H, dd, J = 4.6, 10.5 Hz, H-3α ), 1.67 (1H, brs, Me-30), 1.13 (3H, brs, Me-23), 0.97 (3H, brs, Me-25), 0.91 (3H, brs, Me-26), 0.84 (3H, brs, Me-24), 0.76 (3H, brs, Me-27), 2.63-1.51 (25H, m, 10 x $CH_2$ , 5 x CH); $^{13}C$ NMR ($CDCl_3$ ): δ 38.85 (C-1), 27.13 (C-2), 79.03 (C-3), 40.18 (C-4), 56.33 (C-5), 18.28 (C-6), 34.31 (C-7), 40.99 (C-8), 50.48 (C-9), 37.29 (C-10), 20.84 (C-11), 25.48 (C-12), 38.36 (C-13), 42.43 (C-14), 27.98 (C-15), 30.67 (C-16), 55.31 (C-17), 49.22 (C-18), 46.12 (C-19), 150.52 (C-20), 29.70 (C-21), 35.19 (C-22), 32.16 (C-23), 20.65 (C-24), 16.12 (C-25), 15.56 (C-26), 14.68 (C-27), 181.31 (C-28), 110.31 (C-29), 22.70 (C-30); +ve FAB MS *m/z* (rel. int.): 456 $[M]^+$ ($C_{30}H_{48}O_3$) (100), 441 (18.2), 438 (12.1), 410 (17.5).

**6-O-α-D-Glucosyl α-D-glucose (6)**

Elution of the column with ethyl acetate – methanol (9:1) afforded colourless powder of **6**, recrystallized from ethanol, 213 mg, $R_f$ : 0.86 (*n*-butanol – acetic acid-water, 5:4:1); m. p. 160 - 162 °C, IR $\upsilon_{max}$ (KBr): 3571, 3175, 2925, 2845, 1432, 1385, 1260, 851 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 5.22 (1H, d, J = 5.1 Hz, H-1α), 4.82 (1H, m, H-5), 4.43 (1H, dd, J = 6.0, 6.3 Hz, H-2), 3.76 (1H, m, H-3), 3.49 (1H, m, H-4), 3.39 (1H, d, J = 5.4 Hz, $H_2$-6), 5.06 (1H, d, J = 6.0 Hz, H-1′), 4.77 (1H, m, H-5′), 3.89 (1H, d, J = 5.1, 7.8 Hz, H-2′), 3.54 (1H, m, H-3′), 3.46 (1H, m, H-4′), 3.12 (2H, d, J = 9.0 Hz, $H_2$-6′); $^{13}C$ NMR ($CDCl_3$ ): δ 92.04 (C-1), 74.54 (C-2), 72.65 (C-2), 73.07 (C-3), 71.88 (C-4), 82.72 (C-5), 60.79 (C-6), 104.21 (C-1′), 73.18 (C-2′), 73.07 (C-3′), 70.12 (C-4′), 77.35 (C-5′), 62.39 (C-6′); +ve ESI MS *m/z* (rel. int.): 342 $[M]^+$ ($C_{12}H_{22}O_{11}$) (18.1), 179 (5.2), 163 (100).

## RESULTS AND DISCUSSION

Compound **1** was a known pentacyclic triterpene characterized as davallene (fern-9(11)-ene, fernane).[23,24]

Compound **2**, named 16′-methyl *n*-heptadecanyl protocatechuate, responded positive tests of phenols, showed UV absorption maximum at 297 nm for aromatic ring and IR absorption bands for hydroxyl groups (3274 $cm^{-1}$), ester function (1725 $cm^{-1}$), aromatic ring (1634, 1550 $cm^{-1}$) and long aliphatic chain (727 $cm^{-1}$). On the basis of mass and $^{13}C$ NMR spectra its molecular ion peak was determined at *m/z* 407 $[M+H]^+$ consistent with a molecular formula of an alkylated dihydroxybenzoate, $C_{25}H_{43}O_4$. The ion peaks arising at *m/z* 137 [$C_7$- O

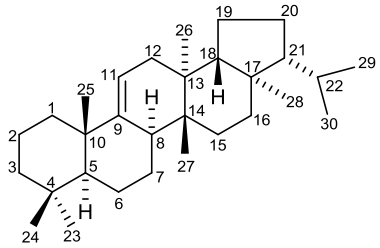
fission, $(HO)_2$-$C_6H_3$-$CO]^+$ , 153 [$C_{1'}$ - O fission, $(HO)_2$-$C_6H_3$-$COO]^+$ and 363 [$C_{15'}$ - $C_{16'}$ fission, $(HO)_2$-$C_6H_3$-$COOCH_2$-$(CH_2)_{14}]^+$ indicated that dihydroxybenzoate was present at one of the end carbon of the ester and isopropyl group at another end of the molecule. The ion fragments generated at *m/z* 269 [M – 137, $C_{18}H_{37}O]^+$ and 253 [M – 153, $C_{18}H_{37}]^+$ suggested attachment of the C-18 alkyloxy group to the aromatic acid. The $^1$H NMR spectrum of **2** displayed two one-proton doublets at δ 6.51 (J = 1.8 Hz) and 6.01 (J = 7.8 Hz) and a one-proton double doublet at δ 6.48 (J = 1.8, 7.8 Hz) assigned to aromatic H-2, H-5 and H-6 protons, respectively. A two-proton triplet at δ 4.01 (J = 8.4 Hz) and a one-proton multiplet at δ 1.81 were ascribed correspondingly to oxymethylene $H_2$-1′ and methine H-16′ protons. Two three-proton doublets δ 0.97 (J = 7.2 Hz) and 0.85 (J = 6.9 Hz) were accounted to terminal C-17′ and C-18′ secondary methyl protons, respectively. The other methylene protons resonated as a four-proton multiplet at δ 1.50 and as a singlet at δ 1.31 (24H). The $^{13}$C NMR spectrum of **2** exhibited signals for ester carbon at δ 173.83 (C-7), aromatic carbons between δ 167.29 – 115.29, oxymethylene carbon at δ 67.31 (C-1′), methine carbon at δ 35.72 (C-16′), methylene carbons from δ 34.52 to 22.61 and methyl carbons at δ 14.82 (Me-17′) and 14.63 (C-18′). Acid hydrolysis of **2** yielded protocatechuic acid, m. p. 219 – 221 $^o$C. On the basis of the aforementioned spectral data analysis and chemical reactions the structure of **2** has been elucidated as 16′-methyl *n*-heptadecanyl 3,4-dihydroxybenzoate, a new aromatic ester (Fig. 1).

Compound **3** responded positively to Liebermann-Burchard test suggesting its triterpenic nature. Its IR spectrum exhibited absorption bands for hydroxyl group (3410 cm$^{-1}$). On the basis of mass and $^{13}$C NMR spectra its molecular ion peak was determined at *m/z* 429 $[M+H]^+$ consistent with a molecular formula of a saturated pentacyclic triterpenol, $C_{30}H_{53}O$. The ion peaks generating at *m/z* 413 [M - Me]$^+$, 398 [413 - Me] $^+$ and 410 [M - $H_2O$] $^+$ suggested the existence of methyl and hydroxyl groups in the molecule. The $^1$H NMR spectrum of **3** showed a one-proton double doublet at δ 3.73 (J = 5.3, 5.8 Hz) assigned to β-oriented carbinol H-3 proton, six three-proton broad singlets at δ 1.25, 0.97, 0.84, 0.81, 0.79 and 0.68 ascribed to tertiary C-23, C-27, C-24, C-26, C-25 and C-28 methyl protons, respectively, two three-proton doublets at δ 1.17 (J=6.3 Hz) and 0.99 (J=7.5 Hz) attributed correspondingly to secondary C-29 and C-30 methyl protons and the remaining methine and methylene protons in the range of δ 2.40 – 1.28. The presence of all the methyl signals between δ 1.25 - 0.68 indicated their attachment on the saturated carbons. The $^{13}$C NMR spectrum of **3** showed 30 carbon signals in the molecule. The C-3 carbinol carbon appeared at δ 72.84 ppm. The carbon signals at δ 33.41 (C-23), 14.13 (C-24), 21.60 (C-25), 21.75 (C-26), 16.72 (C-27), 15.86 (C-28), 15.21 (C-29), 16.75 (C-30) were assigned to methyl carbons. The absence of $^1$H NMR signals beyond 3.73 ppm and $^{13}$C NMR signals from 72.84 ppm supported saturated nature of the molecule. The DEPT spectrum of **3** showed the presence of eight methyl, ten methylene, seven methine and five quaternary carbons. The $^1$H-$^1$H COSY spectrum of **3** exhibited correlation of H-3 with $H_2$-1, $H_2$-2, H-5 and $H_3$-23; H-9 with H-8, $H_2$-7 and $H_2$-11; and H-21 with $H_2$-20, H-22, $H_3$-29 and $H_3$-30. The HMBC spectrum of **3** displayed interactions of $H_2$-1, $H_2$-2, H-5 and $H_3$-24 with C-3; $H_2$-12, H-18 and $H_3$-26 with C-13; and H-21, $H_3$-29 and $H_3$-30 with C-22. The $^1$H and $^{13}$C NMR spectral values of **3** were compared with the fernane-type triterpenoids.[25-27] On the basis of these evidences, the structure of **3** has been established as 3-α-hydroxyfernane (farnan-3α-ol), a rare pentacyclic triterpenol.

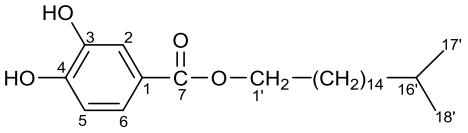
Compound **4** gave positive Liebermann-Burchard test for triterpenes and showed characteristic IR absorbance bands for hydroxyl groups (3415, 3383 cm$^{-1}$). On the basis of mass and $^{13}$C NMR spectra the molecular ion peak of compound **4** was determined at *m/z* 445 $[M+H]^+$ corresponding to the molecular formula of dihydroxy pentacyclic triterpene $C_{30}H_{53}O_2$. The ion peaks arising at *m/z* 140 [$C_{5,6}$- $C_{9,10}$ fission, $C_9H_{16}O]^+$, 194 [$C_{8,14}$- $C_{9,11}$ fission, $C_{13}H_{22}O]^+$, 250 [M - 194]$^+$ and 276 [$C_{14,15}$- $C_{13,18}$ fission, $C_{19}H_{32}O]^+$ indicated the existence of one of the hydroxy group in ring A placed at C-3 on the basis of biogenetic consideration and saturated nature of rings A-D. The ion peaks produced at *m/z* 168 [M – 276, $C_{11}H_{20}O]^+$ and 124 [$C_{16,17}$ - $C_{13,18}$ fission, $C_9H_{16}]^+$ suggested the presence of another hydroxy group in ring D at C-16 carbon, saturated nature of ring E and isopropyl group in ring E. The $^1$H NMR spectrum of the **4** exhibited two one-proton double doublets at δ 3.72 (J = 5.2, 8.8 Hz) and 3.63 (J = 5.5, 8.6 Hz) assigned to α-oriented carbinol H-3 and H-16 protons, respectively. Six three-proton broad singlets at δ 1.25, 0.94, 0.84, 0.81, 0.79 and 0.60 and two three-proton doublets at δ 1.15 (J = 6.3 Hz) and 0.96 (J = 5.5Hz) were ascribed correspondingly to tertiary C-23, C-27, C-24, C-25, C-26, C-28 and secondary C-30 and C-29 methyl protons, all of them attached to saturated carbons. The remaining methine and methylene protons appeared as multiplets between δ 2.37 – 1.31 (24H). The $^{13}$C NMR spectrum of the compound **4** displayed thirty carbon signals including two carbinol carbons at δ 72.84 (C-3) and 74.01 (C-16) and methyl carbons between δ 33.25 – 14.13. The DEPT spectrum of the compound **4** showed the presence of eight methyl, nine methylene, eight methine and five quaternary carbons. The absence of any signal beyond δ 3.72 in the $^1$H NMR spectrum and beyond δ 74.01 in the $^{13}$C NMR spectrum supported the saturated nature of the compound **4**. The $^1$H and $^{13}$C NMR spectral data of compound **4** were compared with the reported spectral data of farnane-type triterpenoids. [25-27] On the basis of afore-mentioned discussion, the structure of compound **4** has been formulated as farnan-3β, 16β-diol, a rare pentacyclic triterpene diol (Fig. 1).

Compound **5** was a known lupeol-type pentacyclic triterpenic acid characterized as betulinic acid (Fig. 1).[28-

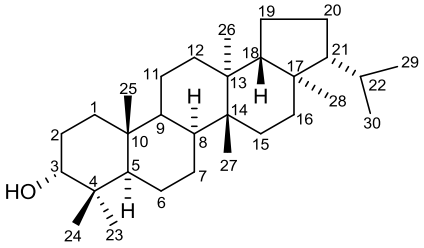
[30] Compound **6**, $[M]^+$ at *m/z* 342 ($C_{12}H_{22}O_{11}$), was a known α-D-diglucoside identified as α-D-glucopyranosyl-(6→1′)-O- α-D-glucopyranoside.[31]



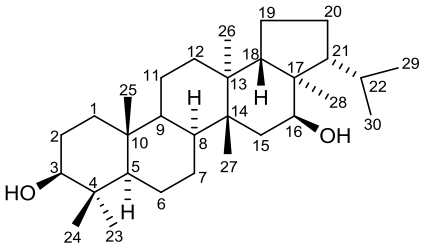
**Davallene (1)**



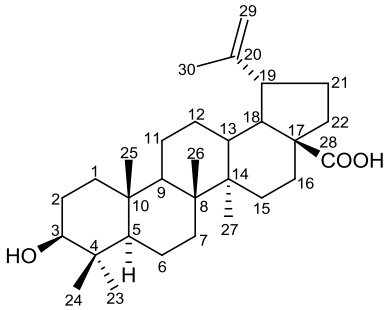
**16′-Methyl *n*-heptadecanyl protocatechuate (2)**



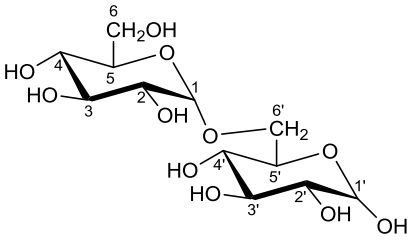
**Farnan-3α-ol (3)**



**Farnan-3β, 16β-diol (4)**



**Betulinic acid (5)**



**α-D-(1→ 6′)-Diglucoside (6)**

**Fig. 1: Structural formulae of the chemical constituents 1 – 6 isolated from the fronds of *Adiantum capillus- veneris***

## CONCLUSION

Phytochemical investigation of the fronds of *Adiantum capillus- veneris* led to the isolation of four pentacyclic triterpenes characterized as davallene (fern-9(11)-ene, **1**), 3-α-hydroxyfernane (farnan-3α-ol, **3**), farnan-3β, 16βdiol (**4**) and betulinic acid (**5**), an aromatic ester identified as 16′-methyl n-heptadecanyl 3,4- dihydroxybenzoate (**2**) and a rare diglucoside α-Dglucopyranosyl-(6→1′)-O- α-D-glucopyranoside (**6**). This work has enhanced understanding about the phytoconstituents of the undertaken plant. These secondary metabolites can be used as analytical markers for quality control of the fern.

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## REFERENCES

1. Anonymous. The wealth of India. Volume- I: A. New Delhi: National institute of Science Communication and Information Resources (CSIR), 2003; 79-80
2. Duke JA, Bogenschutz-Godwin MJ, Du Celliar J, Duke PAK. *Adiantum capillus-veneris* L. In: Handbook of medicinal herbs, 2nd ed. Boca Raton, CRC Press, 2002.
3. Kirtikar KR and Basu BD. Indian medicinal plants with illustration. 2nd ed. Dehradun, International Book Distributors, 2003; 3747-3749.
4. Quattrocchi, U. CRC World Dictionary of Medicinal and Poisonous Plants: Common names, scientific names, eponyms, synonyms, and etymology, CRC Press, Boca Raton, Florida, 2012; 88 – 89.
5. Sato N, Furuya M. Isolation and identification of diacylglyceryl-O-4′-(N,N,N-trimethyl)- homoserine from the fern *Adiantum capillus-veneris* L. Plant and Cell Physiology, 1983; 24(6): 1113–1120. https://doi.org/10.1093/oxfordjournals.pcp.a076615.
6. Nakane T, Arai Y, Masuda K, Ishizaki Y, Ageta H, Shiojima K. Fern constituents : six new triterpenoid alcohols from *Adiantum capillus-veneris*, Chemical and Pharmaceutical Bulletin, 1999; 47(4): 543-547. https://doi.org/10.1248/cpb.47.543

7. Ibrahim ZZ, Ahmed AS and Gouda YG. Phytochemical and biological studies of *Adiantum capillus-veneris* L. Saudi Pharmaceutical Journal, 2011; 19(2): 65-74; https://doi.org/10.1016/j.jsps.2011.01.007.
8. Haider S, Kharbanda C, Alam MS, Hamid H, Ali M, Alam M, Nazreen S, Ali Y. Anti- inflammatory and anti-nociceptive activities of two new triterpenoids from *Adiantum capillus-veneris* Linn. Natural Product Research, 2013; 27(24): 2304-2310, DOI: 10.1080/14786419.2013.828292.
9. Shiojima K, Arai Y, Masuda K, Takase Y, Ageta T and Ageta H. Mass spectra of pentacyclic triterpenoids. Chemical & Pharmaceutical Bulletin, 1992; 40: 1683-1690.
10. Abdel-Halim OB, Ibraheim ZZ and Shiojima K. Oleanane triterpenes from *Adiantum capillus-veneris* growing in Egypt. Alexandria Journal of Pharmaceutical Sciences, 2002; 6: 87-92.
11. Nakane T, Maeda Y, Ebihara H, Arai Y, Masuda K, Takano A, Ageta H, Shiojima K, Cai S-Q and Abdel-Halim OB. Fern constituents: triterpenoids from *Adiantum capillus- veneris*. Chemical & Pharmaceutical Bulletin (Tokyo), 2002; 50(9): 1273-1275. doi: 10.1248/cpb.50.1273.
12. Mahran G.H., El-Alfy T.S., El-Tantawy M.M., El-Sakhaw Y.F. A Contribution to the of chemical constituents of *Adiantum capillus-veneris* L. growing in Egypt. Al-Azhar Journal of Pharmaceutical Science, 1994; 13: 1–14.
13. Imperato F. Sulphate esters of hydroxycinnamic acid-sugar derivatives from *Adiantum capillus-veneris*. Phytochemistry, 1982; 21: 2717-2718.
14. Imperato F. Kaempferol 3-sulphate in the fern *Adiantum capillus-veneris*. Phytochemistry, 1982; 21: 2158-2159.
15. Imperato F. 1982. New phenolic glycosides in the fern *Adiantum capillus-veneris* L. Chemistry & Industry, 1982; 23: 957 - 958.
16. Rajurkar NS and Gaikwad K. Evaluation of phytochemicals, antioxidant activity and elemental content of *Adiantum capillus veneris* leaves. Journal of Chemical and Pharmaceutical Research, 2912; 4(1): 365-374.
17. El-Tantawy M, El-Sakhawy F, El-Deeb K, Fathy M, Hassan A-K. A phytochemical and pharmacological study of *Adiantum capillus veneris* L. growing in Egypt. Zagazig Journal of Pharmaceutical Sciences, 1994; 3: 97-103.
18. Hussein HM, Hameed IH, Ibraheem OA. Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of *Adiantum capillus-veneris* using GC-MS and FT- IR spectroscopy. International Journal of Pharmacognosy and Phytochemical Research, 2016; 8(3): 369-385.
19. Khodaie L, Esnaashari S, Moghaddam SB. Essential oil of arial parts of *Adiantum capillus-veneris*: chemical composition and antioxidant activity. Jundishapur Journal of Natural Pharmaceutical Products, 2015; 10(4): e219682.
20. Fonsa F, Froissard D, Bessière J-M, Buatoisc B, Rapior S. Biodiversity of volatile organic compounds from five French ferns , Natural Product Communications, 2010; 5(10): 1655 – 1658.
21. Marino A, Elberti MG, Cataldo A. Phytochemical investigation of *Adiantum capillus- veneris*. Bollettino della Societa Italiano di Biologia Sperimentale, 1989; 65(5): 461- 463.
22. Kumar SS, Samydurai P., Ramakrishnan R., Nagarajan N. Gas chromatography and mass spectrometry analysis of bioactive constituents of *Adiantum capillus-veneris* L., International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(4): 60—63.
23. Reddy VLN, Ravikanth V, Rao TP, Diwan PV, Venkateswarlu Y. A new triterpenoid from the fern *Adiantum lunulatum* and evaluation of antibacterial activity. Phytochemistry, 2001; 56: 173-175; DOI: 10.1016/s0031-9422(00)00334-4.
24. Jankowski CK, Aumelas A, Thuery P, Reyes-Chilpa R, Jimenez-Estrada M, Barrios H, Diaz E. Xray, 1H/13C 2D and 3D NMR studies of the structures of davallene and adipedatol, two triterpenes isolated from *American Adiantum capillus-veneris*. Polish Journal of Chemistry, 2004; 78: 389- 408.
25. Ali M.: Techniques of Terpenoid Identification, Birla Publishers, Delhi, 2001; 431-440.
26. Mukhtar HM, Ansari SH, Ali M, Naved T. New oleanene and fernane-type triterpenes from the stem bark of *Betula pendula* Roth. Pharmazie, 2003; 58(9): 671 – 673.
27. Akhtar N, Ali M, Alam MS. Pentacyclic triterpenes from the stem bark of *Mimusops elengi* L. Acta Poloniae Pharmaceutica - Drug Research, 2008; 66(5): 549 -552.
28. Chandramu C, Manohar RD, Krupadanam DGL, Dashavantha RV. Isolation, characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L. Phytotherapy Research, 2003; 17(2): 129-134. https://doi.org/10.1002/ptr.1088.
29. Bisoli E, Garcez WS, Hamerski L, Tieppo C, Garcez FR. Bioactive pentacyclic triterpenes from the stems of *Combretum laxum*. Molecules, 2008; 13: 2717-2728. DOI: 10.3390/molecules13112717.
30. Sultana S, Ali M, Mir SR, Ahmad D. Lupene triterpenoids from the stem bark of lebbeck, leaves of *Leptospermum scoparium* and roots of *Nardostachys jatamansi*. Acta Scientific Pharmaceutical Sciences, 2017; 1(3): 06-16.
31. Ali M, Singh V, Sultana S, Mir SR. Chemical constituents from the rhizomes of *Alpinia officinarum* and stem barks of *Balanites aegyptiaca* and *Bombax ceiba*. European Journal of Pharmaceutical and Medical Research (EJPMR), 2020; 7(5): 480-490.