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Research Article

A Triterpenic Constituent from the Aerial Parts of *Ardisia Thyrsiflora* D.Don

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Abstract

Ardisia thyrsiflora D. Don (Myrsinaceae, Primulaceae) is a shrub or small tree found in tropical evergreen and semi-evergreen forests in north-eastern India , Nepal, China, Myanmar and Vietnam. The ripe fruits are edible; young leaves are eaten as a vegetable and the plant is used as a post-partum remedy. Phytochemical investigation of a methanolic extract of the aerial parts of A. thyrsiflora resulted in the isolation of a new ursene-type triterpenol characterized as urs-5,12-dien-3α-ol together with stigmasterol and β-D-glucopyranosyl–(4→1′)-O-β-D-glucuronoside. The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions. 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 pure

Keywords: *Ardisia thyrsiflora, aerial parts, urs-5,12-dien-3α-ol, stigmasterol, glucosyl-(4→1')-glucuronoside, characterization*

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Introduction

Ardisia thyrsiflora D. Don, syn. *A. floribunda* Wall., *A. nerifolia* Wall. ((Myrsinaceae, Primulaceae), commonly known as laungla-chunt, Thengpi-charleng and Nan fang zi jin, is a shrub or small tree with entire leaves, reddish flowers and red-dotted depressed berries. It is found in tropical evergreen and semi-evergreen forests, woody hillsides, valleys, damp places and thickets between 200 – 1500 m in north-eastern India , Nepal, China, Myanmar and Vietnam. The ripe fruits are edible; young leaves are eaten as a vegetable. The plant is used as a post-partum remedy [1]. The other *Ardisia* species are used to treat a variety of diseases. For example, *A. colorata* Roxb. Is useful to cure cough, diarrhoea, lameness, lumbago and rheumatism [2] , *A. crassa* C.B.Clarke is prescribed to relieve rheumatism [2] and *A. elliptica* Thunb. is recommended to alleviate pain associated with the heart [2], ear pain, fever and diarrhea [3]. The plant *A. floribunda* contained flavones characterized as 5, 6, 3', 4'-tetramethoxyflavone and 5,6, 2', 3', 4'-pentamethoxyflavone [4]. A triterpenoid saponin, ardisikivuoside identified as 3-O-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D- ylopyranosyl-3β-hydroxy-13β,28-epoxyoleanan-16-oxo-30-al was isolated from *A. kivuensis* [5]. The present paper describes isolation and characterization of chemical constituents from the aerial parts of *A. thyrsiflora*.

Materials and Methods

General procedures

Melting points were determined on a Perfit apparatus without correction. The IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer. The ^{1}H (400 MHz), ^{13}C (100 MHz) NMR spectra were recorded on Bruker spectrospin spectrometer. $CDCl_3$ and DMSO-d_6 (Sigma-Aldrich, Bangalore, India) were used as solvents and TMS as an internal standard. ESI MS analyses were performed on a Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK) Mass Spectrometer. Column chromatography separations were carried out on silica gel (Merck, 60–120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60 F_{254}) were used for analytical thin layer chromatography and the spots were visualized by exposure to iodine vapours and UV radiations.

Plant material

The aerial parts of *A. thyrsiflora* were collected from a field of Manipur and identified by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi.

Extraction and isolation

The air-dried coarsely powdered aerial parts (2 kg) were extracted exhaustively in a Soxhlet apparatus with methanol for 72 hr. The methanolic extract was concentrated under reduced pressure to obtain dark brown viscous mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous dark brown mass was adsorbed on silica gel (60-120 mesh) for column chromatography, after being dissolved in little quality of methanol for preparation of slurry. The slurry (200 g) was air dried and subjected to chromatography over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1, and 1:3), chloroform and the mixture of chloroform and methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3). Various 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 pure

Urs-5,12-dien-3α-ol

Elution of the column with chloroform gave colourless powder of **2**, recrystallized from acetone, 312 mg, m. p. 236-237 ^{0}C, UV λ_{max} (MeOH): 207 nm (log ε 3.8); IR γ_{max} (KBr): 3405, 2924, 2852, 1645, 1461, 1379, 1217, 1015, 980 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ δ 5.37 (1H, m, H-6), 5.18 (1H, dd, J = 6.2, 11.2 Hz, H-12), 3.15 (1H, dd, J=4.8, 5.2 Hz, H-3β), 2.18 (1H, d, J = 5.11 Hz, H-18β), 1.06 (3H, brs, Me-24), 1.01 (3H, d, J = 6.5 Hz, Me-29), 0.97 (3H, J = 7.5 Hz, Me-30), 0.93 (3H, brs, Me-27), 0.91 (3H, brs, Me-23), 0.88 (3H, brs, Me-28),), 0.86 (3H, brs, Me-25), 0.65 (3H, brs, Me-26), 2.12 – 1.20 (20H, m, 8 x CH_2; 4 x CH); ^{13}C NMR ($CDCl_3$): δ 38.35 (C-1), 31.26 (C-2), 81.14 (C-3), 38.42 (C-4), 145.39 (C-5), 117. 02 (C-6), 35.32 (C-7), 39.57 (C-8), 47.33 (C-9), 37.78 (C-10), 23.28 (C-11), 124.35 (C-12), 139.54 (C-13), 47.67 (C-14), 27. 85 (C-15), 24.26 (C-16), 50.35 (C-17), 58.69 (C-18), 41.31 (C-19), 31.68 (C-20), 35.83 (C-21), 38.56 (C-22), 28.85 (C-23), 25.74 (C-24), 21.48 (C-25), 16.82 (C-26), 21.96 (C-27), 27.06 (C-28), 19.34 (C-29), 15.14 (C-30); ESI MS *m/z* (rel. int.): 424 $[M]^{+}$ ($C_{30}H_{48}O$) (12.8), 272 (23.8), 258 (14.8), 220 (13.8), 218 (14.2), 205 (23.7), 203 (23.1), 187 (31.7), 172 (22.5), 159 (41.9), 152 (62.9), 134 (45.2).

Stigmasterol (2)

Further elution of the column with chloroform yielded a colourless amorphous powder of **2**, 120 mg (0.12 % yield), m. p. 166-168 $^{\circ}C$; UV λ_{max} (MeOH): 211 nm (log ε 5.8); IR γ_{max} (KBr): 3425, 2920, 2852, 1641, 1463, 1373, 1225, 1173, 801 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.36 (1H, m, H-6), 5.16 (1H, m, H-22), 5.01 (1H, m, H-23), 3.65 (1H, brm, $w_{1/2}$= 16.5 Hz, H-3α), 2.23 to 1.23 (25 H, m, 9 x CH_2. 7 x CH), 1.05 (3H, brs, Me-19), 0.96 (3H, d, J = 6.3 Hz, Me-21), 0.87 (3H, d, J = 6.6 Hz, Me-26), 0.84 (3H, d, J = 6.0 Hz, Me-27), 0.80 (3H, t, J = 6.6 Hz, Me-29), 0.71 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 36.52 (C-1), 31.90 (C-2), 71.81 (C-3), 41.90 (C-4), 140.76 (C-5), 121.69 (C-6), 31.66 (C-7), 33.94 (C-8), 51.24 (C-9), 37.26 (C-10), 21.07 (C-11), 39.76 (C-12), 42.30 (C-13), 56.87 (C-14), 24.17 (C-15), 28.67 (C-16), 55.96 (C-17), 12.24 (C-18),

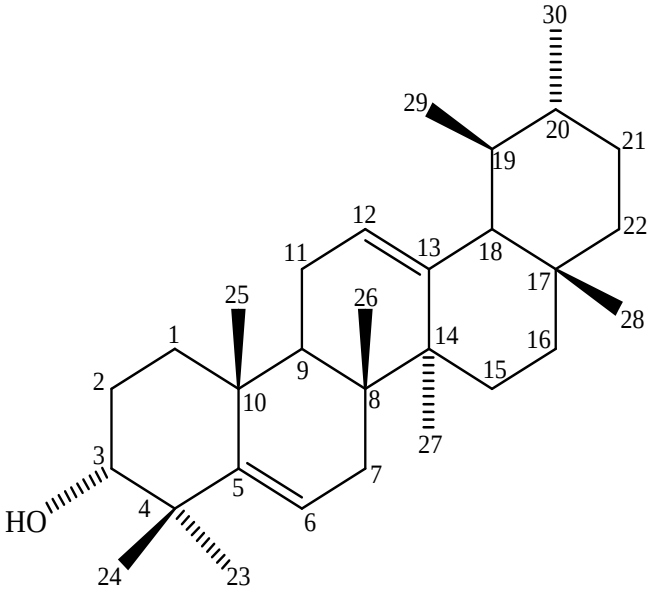
19.41 (C-19), 36.68 (C-20), 18.79 (C-21), 138.30 (C-22), 129.28 (C-23), 45.83 (C-24), 27.28 (C-25), 19.83 (C-26), 18.99 (C-27), 23.11 (C-28), 12.05 (C-29), EIS MS *m/z* (rel. int.): 412 $[M]^+$ ($C_{29}H_{48}O$) (30.2), 394 (50.19), 271 (74.1), 255 (14.8), 220 (33.1), 213 (18.9), 119 (12.7).

Glucosyl-(4→1′)-glucuronoside (3)

Elution of the column with chloroform-methanol (4 : 1) afforded colourless semisolid mass of **3,** recrystallized from methanol, 130 mg; UV λ_{max} (MeOH): 211 nm (log ε 4.2); IR γ_{max} (KBr): 3394, 3308, 3213, 2927, 2842, 1698, 1635, 1456, 1374, 1351, 1342, 1252, 1127. 1092, 993, 837 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.15 (1H, d, J = 7.8 Hz,H-1), 4.37 (1H, m, H-5), 3.64 (1H, m, H-2), 3.55 (1H, m, H-3), 3.38 (1H, m, H-4), 3.18 (2H, d, J = 9.0 Hz, H_2-6), 5.08 (1H, d, J = 7.6 Hz, H-1′), 4.03 (1H, m, H-5′), 3.59 (1H, m, H-2′), 3.35 (1H, m, H-3′), 3.31 (1H, m, H-4′); ^{13}C NMR (DMSO-d6); δ 104.39 (C-1), 70.82 (C-2), 68.54 (C-3), 74.63 (C-4), 77.19 (C-5), 61.36 (C-6), 107.41 (C-1′), 72.89 (C-2′), 70.51 (C-3′), 67.10 (C-4′), 75.74 (C-5′), 170.61 (C-6′). ESI MS *m/z* (rel. int.): 356 $[M]^+$ ($C_{12}H_{20}O_{12}$) (7.6), 193 (11.7), 179 (8.2), 163 (15.1).

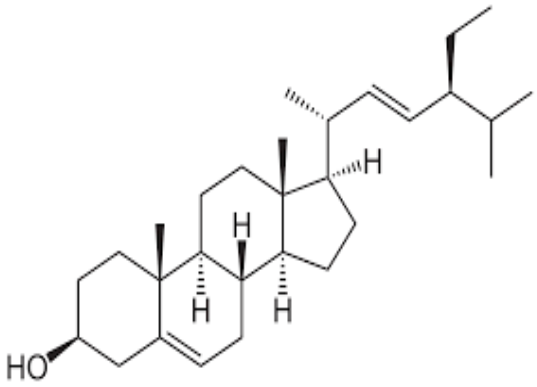
Results and Discussion

Compound **1** responded positively to Liebermann-Burchardt test for triterpenoids and showed IR characteristic absorption bands for hydroxy groups (3405 cm^{-1}) and unsaturation (1645 cm^{-1}). Its molecular ion peak was determined at m/z 424 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of a pentacyclic triterpenoid $C_{30}H_{48}O$. The characteristic ion fragments arising at *m/z* 205 and 218 generated due to retro-Diels Alder fragmentation suggested Δ^{12} olefinic linkage in ring C [6]. Other fragment generating at *m/z* 152, 272 [$C_{14,15}$ – $C_{13,18}$ fission]$^+$, 134 [152 - H_2O]$^+$ and 203 [218 – Me]$^+$ supported the presence vinylic linkages in rings B and C and the hydroxyl function in ring A which was placed at C-3 on the basis of biogenetic considerations. The ion fragments arising at *m/z* 187 [205-H_2O]$^+$, 172 [187-Me]$^+$ and 159 [174-Me]$^+$ also suggested the location of the hydroxyl group in the ring A/B. The ^1H NMR spectrum of **1** displayed two downfield one-proton signals as a multiplet at δ doublet at δ 5.37 and as a double doublet at δ 5.28 (*J* = 6.3, 11.2 Hz) assigned to vinylic H-6 and H-12 protons, respectively. A one-proton double-doublet at δ 3.15 with coupling interactions of 4.8 and 5.2 Hz were ascribed to carbinol H-3β proton. A one-proton doublet at δ 2.18 (J = 5.11 Hz) was due to H-18β proton. The methylene protons resonated in the range from 1.30-1.88. Six three-proton broad singlets at δ 0.87 (Me-23), 1.06 (Me-24), 0.86 (Me-25), 0.65 (Me-26), 0.93 (Me-27) and 0.88 (Me-28) and two three-proton doublets at δ 1.01 (*J* = 6.5 Hz, Me-29) and 0.97 (J = 7.5 Hz, Me-30) were attributed to methyl protons of ursine-type compound. The remaining methine and methylene protons appeared from δ 2.12 to 1.20. The ^{13}C NMR spectrum of **1** displayed signals for thirty carbons and important were appeared due to vinylic carbons at δ 145.39 (C-5), 117. 02 (C-6), 124.35 (C-12) and 139.54 (C-13), carbinol carbon at δ 81.14 (C-3) and methyl carbons from δ 28.85 to 15.14. The assignments of the carbon chemical shift were made by comparison with δ values of corresponding carbon atom of urs-12-enes [6-8]. On the basis of above discussion the structure of **1** was elucidated as urs-5,12-dien-3α-ol. This is a new pentacyclic triterpene.



Urs-5,12-dien-3α-ol **(2)**

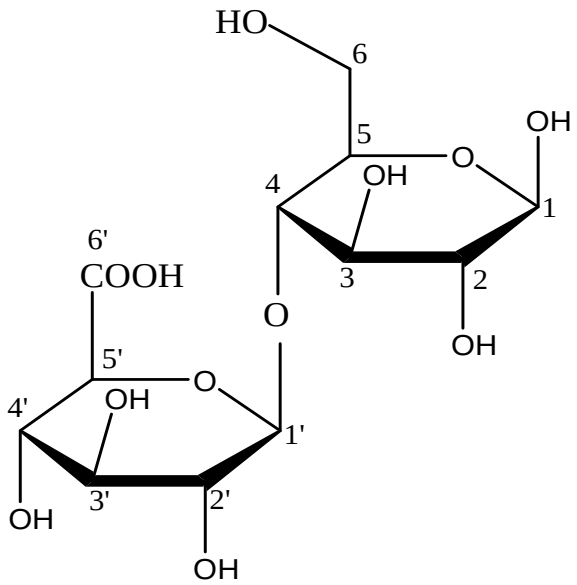
Compounds **2** was the known phytoconstituent characterized as stigmasterol [9,10] .



Stigmasterol (**2**)

Compound **3**, designated as glucosyl-(4→1′)-glucuronoside, was obtained as a colourless semisolid mass from chloroform-methanol (4:1) eluants. It gave positive tests for glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3394, 3308, 3213 cm^{-1}) and carboxylic function (1698 cm^{-1}). The mass spectrum of **3** exhibited the molecular ion peak at *m/z* 356 corresponding to a molecular formula of a diglycoside, $C_{12}H_{20}O_{12}$. The ion fragments gen-

erating at *m/z* 193 $[C_6H_9O_7]^+$, 179 $[C_6H_{11}O_6]^+$ and 163 $[C_6H_{11}O_5]^+$ indicating that a hexose unit C_6 sugar acid. The ^{1}H NMR spectrum of **3** exhibited two one-proton doublets at δ 5.15 (J = 7.8 Hz) and δ 5.08 (J = 7.1 Hz) assigned to anomeric H-1 and H-1′ protons, respectively. Eight one-protons multiplets between δ 4.37- 3.31 were ascribed to carbinol protons of the sugar unit. A two-proton doublet at δ 3.18 (J = 9.0 Hz) was accounted to hydroxymethylene H_2-6 protons. The ^{13}C NMR spectrum of **3** exhibited signals for the carboxylic carbon at δ 170.61 (C-6′), anomeric carbons at δ 104.39 (C-1′) and δ 107.41 (C-1″) and other sugar carbons in the range from δ 77.19 to 61.36. The presence of a ^{13}C NMR signal for C-4 at δ 74.63 in the deshielded region suggested the attachment of another sugar by a (4→1′) linkage. Acid hydrolysis of **3** yielded D-glucose (R_f 0.12, *n*-butanol-acetic acid-water, 4:1:5) and D-glucuronic acid (R_f 0.38, *n*-butanol-methanol-water, 5:2:1). On the basis of the above discussion the structure of **3** has been elucidated as *β*-D -glucopyranosyl–(4→1′)-O-β-D-glucuronoside.



Glucosyl-(4→1')-glucuronoside (**3)**

Conclusion

Phytochemical investigation of a methanolic extract of the aerial parts of *A. thyrsiflora* resulted in the isolation of a new ursene-type triterpenol characterized as urs-5,12-dien-3α-ol together with stigmasterol and *β*-D-glucopyranosyl–(4→1′)-O-β-D-glucuronoside.

This work has enhanced understanding about the phytoconstituents of the plant. These compounds may be used as chromatographic markers for standardization of the aerial parts.

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Declaration of Interest

The authors report no conflicts of interest.

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