Phytochemical Analysis of Leaves of *Ardisiasolanacea*Roxb.

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Abstract – The ethylacetate extract of air-dried and powdered leaves of *Ardisia solanacea* was subjected to repeated coloumn chromatography (CC) over silicagel eluted with choloroform and methanol ($CHCl_3$: MeOH; 100:0→1:1) afforded various fractions which on repeated column chromatography over silica geleluted with different solvents yielded β-sitosterol, gallic acid, Quercetin, Myricetin and a new alkylphenolic compound identified as (-)-5-(1,2-Dihyd-roxypentyl)benzene-1,3-diol.
Identification of these compounds were made on the basis of analysis of their physical and spectroscopic data and chemical methods.

Key words:*Ardisiasolanacea*, Ethyl acetate extract, Alkylphenol, Flavanoids.

Introduction

The *Ardisia* is the largest genus of *Myrsinaceae* family, consisting of approximately 500 species of evergreen shrubs and trees found throughout the subtropical and tropical regions of the world[1]. *Ardisia* species were used in traditional medicines inimprovement of liver cancer, swelling, rheumatism, earache, cough, fever, diarrhea, inflammation, respiratory tract infection, traumatic injury, broken bone, pain, snake and insect bite, birth complications and to improve general blood circulation[2]. Diverse types of compounds have been isolated from this genus, such as polyphenols, triterpenoid saponins, coumarins, quinones, flavonoids and alkylphenols[3]. *Ardisia* species possess very important biological activities like anti-oxidant, analgesic, utero-contraction, anti-platelet, cytotoxic, anti-inflammatory, cAMP inhibiting, anti-feedant, anti-thrombin, hepato protective, antitumour, antibiotic, antiviral, anti-allergic and anti-HIV activities[3].
*Ardisiasolanacea*Roxb.,have been reported to possess stimulant and carminative properties and used as anti-acetlycholine, in internal injury, stomachache especially after childbirth, as a febrifuge, in diarrhoea and in rheumatism[4-5]. Triterpenoids, alcohols, bauerenol, α-amyrin and β-amyrin have been reported so far from the leaves of*A. solanacea*[6]. In the present study the phytochemical analysis of ethyl acetate extract of air dried leaves of *A. Solanacea* was carried out using column chromatography over Si-gel using various solvents afforded β-sitosterol, gallic acid, Quercetin, Myricetin and a new alkylphenolic compound identified as (-)-5-(1,2Dihydroxypentyl)benzene1,3diol(1). Identification of these compounds was made by physical and spectroscopic techniques.

Material and Methods

CC was carried out over silica gel (60-120 mesh BDH) using gradient elution with different solvent systems in order of increasing polarity. TLC was carried out on Silica-gel (E-Merck and BDH) coated on a thin glass plate (0.25 mm thickness containing 13% $CaSO_4$ as binder). Spots on TLC were detected by spraying with

5% H_2SO_4 followed by heating at 100^0C, 5% methanolic KOH, Benedict's reagent, iodine vapours, UV and alcoholic $FeCl_3$ solution. Melting points were recorded in BOETIUS microscopic m.p. apparatus. The UV-spectra (λ_{max}, nm) were recorded in Systronic spectrophotometer using MeOH as solvent. The IR-spectra (ν_{max}, cm^{-1}) were recorded using KBr palettes on FT-IR-8100 Shimadzu spectrophotometer and optical rotations were recorded on JASCO DIP-140 digital polarimeter in methanol. NMR spectra were recorded in BRUKER DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrophotometer with DMSO-d_6 and CD_3OD solvents. Chemical shifts are given in ppm scale with TMS as an internal standard. Mass spectra were recorded in JEOLD-300 (EI/CI) spectrometer.

Plant Material: The leaves of *Ardisiasolanacea*were collected from LaxmanSiddhHarawala (Dehradun), Uttarkhand, India, in September 2017. The plant species was identified by Dr. Sumer Chand, Department of Systematic Botany, Forest Research Institute, Dehradun, U.K. A voucher specimen (H.R N0.101) was deposited in the Department of Botany Govt.P.G. College, Uttarkashi, U.K., India.

Extraction and Isolation: The air-dried and powdered leaves (3.5kg) of *A. solanacea* were exhaustively defatted with light petroleum ether ($60\text{-}80^0$). The petroleum free mass was extracted with 80% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, which was successively partitioned with ethyl acetate and n-butanol. The ethyl acetate and n-butenol layer was separated out and concentrated under reduced pressure to give EtOAc extract (42.5g) and n-BuOH extract (20.5g).

The EtOAc extract was found to have more concentration of the phytoconstituents as monitored by TLS, therefore, it was subjected to repeated coloumn chromatography (CC) over Si-gel eluted with $CHCl_3$:MeOH (100:0→1:1) afforded various fractions. The like fractions (monitored by TLC) were mixed together. Fraction **I**, on repeated CC over Si-gel using gradient elution with C_6H_6:EtOAc yielded compound β-sitosterol (51 mg), gallic acid (46mg). Fraction **II**, on repeated CC over Si-gel using gradient elution with $CHCl_3$:MeOH yield edgallic acid (23mg), and an alkylphenol identified as (-)-5-(1,2-dihydroxypentyl)benzene-1,3-diol (**1**)(113mg). Fraction **III**, on repeated CC over Si-gel eluted with gradient elution $CHCl_3$:MeOH, afforded quercetin (51mg) and myricetin (63mg).

Results and Discussion

β-sitosterol(1)
White amorphous solid
M.p. 135-137^{0}C
$[\alpha]_D^{25}$**:** -36^0 (c=0.1, $CHCl_3$)
IR (ν_{max}^{KBr}):3340, 2970, 2959, 2920, 1463 cm^{-1}.

Gallic acid (2) White crystalline solid
M.P. 160-162^{o}C
IR (ν_{max}^{KBr}): cm^{-1} 3492, 2900-2650, 1715 cm^{-1} (-C=O)
^{1}H-NMR(400 MHz, DMSO-d_6): δ 6.93 (2H, *s*, H-2 and H-6), 8.73 (1H, *brs*; C_4-OH), 9.41 (2H, C_3-OH and C_5-OH) and 12.74 (1H, *s*, -COOH)
^{13}C-NMR(100 MHz, DMSO-d_6): δ 121.07 (C-1), 109.45 (C-2,C-6), 144.86 (C-3,C-5), 139.51 (C-4), and 167.64 (-COOH).

(-)-5-(1,2-dihydroxypentyl)benzene-1,3-diol (**3**)
Brown powder
M.p.287-289°C
$[\alpha]_D^{25}$ -15.4 (c=1.0 MeOH)
IR (ν_{max}^{KBr}): cm^{-1} 3600, 2918, 1609, 1585, 1505
HREI-MS: m/z (212) 212.1213 $[M]^+$, $C_{11}H_{16}O_4$ (calc. for 212.1217);
^{1}H-NMR (400 MHz, CD_3OD):δ 6.16 (1H, *t*, *J*=2.2 Hz, H-2), 6.33 (2H, *d*, *J*=2.2

Hz, H-4, 6), 4.35 (1H, *d*, *J*=5.4 Hz, H-1'), 3.63 (1H, *m*, H-2'), 1.52 (1H, *m*, H-3'$_a$), 1.33 (1H, *m*, H-3'$_b$), 1.29 (2H, *m*, H-4') and 0.89 (3H, *t*, *J*=7.0 Hz, H-5')

^{13}C-NMR (100 MHz, CD$_3$OD): δ159.17 (C-1, 3), 102.47 (C-2), 106.69 (C-4, 6), 145.90 (C-5), 78.49 (C-1'), 75.83 (C-2'), 35.27 (C-3'), 19.83 (C-4'), and 14.35 (C-5').

Quercetin (4)

Yellow crystalline solid

M.p. 308-309°C

IR (ν_{max}^{KBr}): cm^{-1} 3289, 3122, 2991, 1660, 1584, 1545, 1457, 1553, 1287, 1205, etc.

HREI-MS: m/z 302.0731 [M]$^+$, (calc. for $C_{15}H_{10}O_7$; 302.0724)

^{1}H-NMR (400 MHz, DMSO):δ 6.19 (1H, *d*, *J*=2.0 Hz, H-6), 6.40 (1H, *d*, *J*=2.0 Hz, H-8), 7.52 (1H, *d*, *J*=2.0 Hz, H-12), 6.81 (1H, *d*, *J*=8.5 Hz, H-15), 7.67 (1H, *dd*, *J*=8.5, 2.0 Hz, H-16), 12.63 (H, *brs*,C_5-OH), 10.85 (1H, *brs*, C_7-OH), 9.72 (1H, *brs*, C_{13}-OH), 9.14 (2H, *brs*, $C_{14,3}$-OH)

^{13}C-NMR (100 MHz, DMSO):δ148.01 (C-2), 136.46 (C-3), 177.47 (C-4), 161.21 (C-5), 98.64 (C-6), 164.10 (C-7), 93.47 (C-8), 158.01 (C-9), 104.20 (C-10), 123.99 (C-11), 115.56 (C-12), 148.81 (C-13), 144.81 (C-14), 116.21 (C-15), 121.67 (C-16).

Myricetin (5)

Yellow powder

M.p. 343-344°C

lR (ν_{max}^{KBr}): cm^{-1} 3390, 2920, 1625, 1600, 1500, 1455, 1368, 1280, 1220, etc.

HREI-MS: m/z 318.0709 [M]$^+$; (calc. for $C_{15}H_{10}O_8$; 318.0713)

^{1}H-NMR (400 MHz, CD$_3$OD):δ 6.11 (1H, *d*, *J*=2.0 Hz, H-6), 6.32 (1H, *d*, *J*=2.0 Hz, H-8), 7.23 (1H, *s*, H-12, 16)

^{13}C-NMR (100 MHz, CD$_3$OD):δ148.21 (C-2), 136.23 (C-3), 176.69 (C-4), 161.87 (C-5), 99.62 (C-6), 165.62 (C-7), 94.45 (C-8), 158.22 (C-9), 104.50 (C-10), 122.43 (C-11), 109.87 (C-12, 16), 146.47 (C-13, 15), 137.67 (C-14).

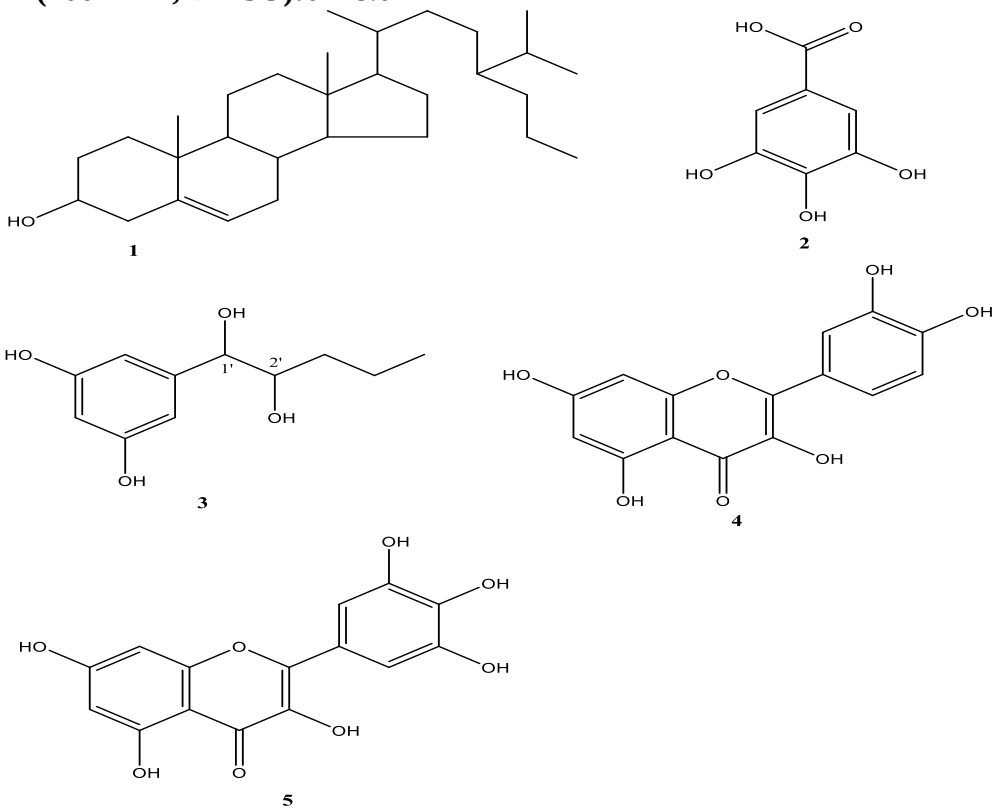

Figure – 1, 2, 3, 4, 5 Isolated Compounds from *Ardisiasolanacea*.

The ethyl acetate extract of air dried leaves of *A. solanacea* on repeated CC over Si-gel afforded a new alkylphenol identified as (-)-5-(1,2-dihydroxypentyl)benzene-1,3-diol (**3**) along with β-sitosterol(**1**), gallic acid (**2**), Quercetin (**4**), Myricetin (**5**). The identification of β-sitosterol[7], gallic acid[8], Quercetin[9] and Myricetin[10-11] was made by direct comparison of their spectral data with the reported data. These compounds were previously isolated from *Ardesia* species[12-18]. Compound **3**was isolated first time from leaves of *A. solanacea* to the best of my knowledge.

Compound**3** obtained as brown amorphous powder. It responded positive to ferric chloride test which indicated the phenolic nature of the compound. The molecular formula of the compound was determined to be $C_{11}H_{16}O_4$ from its HREIMS which showed molecular ion peak at m/z 212.1213 (clac. for 212.1217). Its IR spectrum displayed presence of hydroxyl group at 3600 cm^{-1} and phenyl ring at 2918, 1609 and 1585 cm^{-1}. The ^{1}H NMR spectrum of compound **3** displayed presence of two meta-coupled doublets which indicated the presence of 1,3,5-trisubstituted aromatic ring in the molecule. The chemiscal shift values of these meta coupled doublets at δ 6.16 (1H, *t, J*=2.2 Hz, H-2) and 6.33 (2H, *d*, *J*=2.2 Hz, H-4, 6) in the aromatic region indicated the presence of resorcinol moeity[19]. In aliphatic region the ^{1}H-NMR spectrum showed presence of two signal for methine protons at δ 4.35 (1H, *d*, *J*=5.4 Hz, H-1') and 3.64 (1H, *m*, H-2'). The downfield chemical shifts of these protons indicated that hydroxyl group was present at C-2' and C-3' position. Which was confirmed from downfield chemical shift of carbon atom at δ 78.49 (C-1') and 75.83 (C-2') The ^{1}H-NMR spectrum also displayed signals due to one methyl group at δ 0.89 (3H, *t*, *J*=7.0 Hz, H-5'), and two methylene groups at δ 1.52 (1H, *m*, H-3'$_a$), 1.33 (1H, *m*, H-3'$_b$), and 1.29 (2H, *m*, H-4'). These NMR data indicated the presence of 1,2-dihydroxypentyl side chain in the molecule. The non equivalence of methylene protons (H-3') indicated the presence of chiral centre adjacent to this group.

The ^{13}C NMR spectrum displayed presence eleven carbon atoms whereas DEPT spectrum displayed presence of one methyl, two methylene, four methine (one of double intensity) and two quaterary carbon (one of double intensity atoms). The two equivelent aromatic carbon atoms (C-1, 3) resonated at δ 159.17indicated that the phenolic group was persent at C-1 and C-3 position of phenyl ring which was corroborated with the oxygen carrying carbon of resorcinol. The value of coupling constant (*J* = 5.4 Hz) between H-1' and H-2' protons suggestted that threo configuration of compound **3**.

Conclusion

On the basis of above discussed spectral evidences the structure of compound **3** was determined to be (-)-5-(1,2-dihydroxy-4-methyl-pentyl)benzene-1,3-diol. It was further cofirmed by comparison of spectral data with reported values[20].

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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