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New dimeric and trimeric coumarin glucosides from *Daphne retusa* Hemsl

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ABSTRACT

New dimeric and a trimeric coumarin glucosides namely Daphneretusin A (**1**) Daphneretusin B (**2**) along with three known oligomers (**3**–**5**) were obtained as a result of bioassay guided fractionation of *Daphne retusa* Hemsl. Fractions (*n*-hexane, $CHCl_3$, AcOEt, CH_3OH and water) exhibited potent radical scavenging activity in relevant non-physiological bioassays. The structures of isolated compounds were elucidated by UV, IR, EIMS, FAB-MS, 1D, and 2D NMR spectroscopic analysis.

1. Introduction

Plant family Thymelaeaceae is reputed as a principal source of coumarins, their dimer and other derivatives [1]. It contains economically important members of temperate and tropical regions, comprising of 500 species from 50 genera and *Daphne* is one of them [2]. Various species of *Daphne*, are used in several folk medicines to treat gonorrhea and cutaneous affections [3], wound healings [4], malaria and inflammations [5,6], exhibited few bioactivities that include antimalarial [5], antiviral [7], anti-tumor-promoting [8], antifertility effects [9] antibacterial [10], also regarded as an insecticide and pesticides [11]. Secondary metabolites like coumarins [12,13], flavonoids, diterpenoids [14], lignins [15] and coumarinolignans [16] are reported from the genus *Daphne*. *Daphne retusa* is also among the important species of this genus, found in Himalaya regions of East Asia as well as in northern areas of Pakistan [2]. *D. retusa* is regarded as a traditional folk medicine "Zhu Shi Ma" in China due to detumescence and acesodyne effects [17], while its ethanolic extract (75%) exhibited anti-inflammatory and anti-analgesic activities [18]. According to best of our knowledge until now, fifty coumarins, 19 monomeric coumarins, 22 dimeric-coumarins, and four new trimeric coumarins are reported from genus *Daphne* [19], but only one dimeric and two trimeric coumarins have so far been reported from this species [12,13]. Taking in account the ethno-pharmacological and chemotaxonomic importance of the genus *Daphne* and our interest in medicinal plants of Pakistan [20–25] prompted us to conduct the bioassay guided phytochemical studies of this specie of our region *D. retusa*. In present studies the antioxidant activities of various fractions were established while a few compounds were isolated through bioassay guided isolation.

2. Experimental

2.1. General

Column chromatography (CC) was carried out on silica gel of 70–230 and 230–400 mesh while TLC analysis was conducted on aluminum sheets pre-coated with silica gel 60 F_{254} (20 × 20 cm,

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0.2 mm thick; E-Merck). Purity of the compounds was detected under UV light (Uvitec, model no. LF-204.LS, UK at 254 and 364 nm) followed by spraying reagent ceric sulfate (heating) (Sigma Aldrich). Optical rotations were measured on JASCO DIP-360 digital polarimeter. Melting points were recorded by Gallenkamp apparatus and are uncorrected. The UV spectra were recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm) while IR spectra were recorded on Shimadzu IR-460 spectrophotometer (ν_{max} in cm^{-1}). The ^{1}H-NMR, ^{13}C-NMR, COSY, NOESY, HSQC and HMBC spectra were recorded on Bruker AMX-400 MHz instruments in CD_3OD by using TMS as an internal standard. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (J) in Hz. The HR-FABMS (negative-ion mode) were taken at JMS HX-110 with a data system and JMS DA-500 mass spectrometers, resp.; *m/z* (rel. %).

2.2. Plant material

Fresh *D. retusa* Hemsl (whole plant) was collected from Gilgit (northern areas), Pakistan, in summer season of 2007. The plant was identified by Prof. Manzoor Hussain, Plant Taxonomist, Department of Botany, Government Post Graduate College-1 Abbottabad, Khyber Pakhtonkhaw where a voucher specimen has been deposited in the herbarium (DR-004).

2.3. Extraction and isolation

The freshly collected plant material (whole plant, 26 kg) of *D. retusa* was shade dried (9 kg), ground and extracted with methanol (3 × 15 L, each for 10 days). The combined methanolic extract was evaporated under reduced pressure at room temperature to yield the crude residue (535 g). The whole extract was suspended in water and successively extracted with *n*-hexane (158 g), chloroform (95 g), ethyl acetate (109 g) and water (85 g) soluble fractions. The ethyl acetate soluble fraction was subjected to column chromatography over silica gel and eluted with *n*-hexane, *n*-hexane–chloroform, chloroform and chloroform–methanol in increasing order of polarity to get sub-fractions. The sub-fraction obtained with $CHCl_3$:MeOH (9.0:1.0) was re-chromatographed over silica gel and eluted again with $CHCl_3$:MeOH (9.0:1.0) to provide a semi-pure compound which was finally purified through preparative-TLC with the $CHCl_3$:MeOH (8.5:1.5) eluent to afford compound **1** (13 mg). The sub-fraction obtained with $CHCl_3$:MeOH (8.2:1.8) was re-chromatographed over silica gel and eluted with $CHCl_3$:MeOH (8.9:1.1) to afford compound **2** (9 mg). The sub fraction obtained through elution of DCM:MeOH (1.5:8.5) was subjected to repeated chromatography to yield compounds **3** (12 mg) (DCM:MeOH (7.7:2.3)) and **4** (11 mg) (DCM:MeOH (8.3:1.7)). Similarly sub-fraction afforded through elution of C_6H_{14}:EtOAc (3.0:7.0) solvent system was further subjected to repeated chromatography to afford compound **5** (9 mg, C_6H_{14}:Acetone; 4.0:6.0).

2.4. Daphneretusin A

{6-(β-D-Glucopyranosyl)oxy-7-hydroxy-3-[(2-oxo-2H-1-benzopyran-7-yl)oxy]-2H-1-benzopyran-2-one} (**1**): amorphous powder (13.2 mg); UV: λ_{max} (MeOH): 208 (4.36), 288 (4.16), 326 (4.86), 328 (4.43) nm; IR (KBr) ν_{max}: 3435, 2931, 1718, 1682, 1550, 1502, 1418, 1297, 1155, 998 cm^{-1}; HR-FAB-MS (negative mode): *m/z* 499.3852 $[M-H]^+$, (calcd for $C_{24}H_{19}O_{12}$, 499.2915); EI-MS: *m/z* 338 (98) $[M + H\text{-sugar}]^+$, 310 (14.6), 178 (64.5), 177 (8.2), 165 (47.2), 163 (6.9), 161 (7.2), 151 (5.8), 150 (32); ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) data see Table 1.

2.5. Daphneretusin B

{6-(β-D-Glucopyranosyl)oxy-7-[(7-hydroxy-2-oxo-2H-1-benzopyran-8-yl)oxy]-3-[(2-oxo-2H-1-benzopyran-7-yl)oxy]-2H-1-benzopyran-2-one} (**2**): amorphous powder (9.4 mg); UV: λ_{max} (MeOH): 209 (4.28), 286 (4.21), 328 (4.94), 325 (4.39) nm; IR (KBr) ν_{max}: 3430, 2928, 1722, 1686, 1540, 1499, 1426, 1288, 1199, 989 cm^{-1}; HR-FAB-MS (negative mode): *m/z* 659.4298 $[M-H]^+$, (calcd for $C_{33}H_{23}O_{15}$, 659.3815); EI-MS: *m/z* 497 $[M-C_6H_{10}O_5]^+$, 336 (72), 309 (15.2), 178 (55.2), 177 (10.3), 165 (37.3), 163 (7.7), 151 (8.2); ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) data see Table 1.

2.6. DPPH radical scavenging assay

Spectrophotometric method was employed with certain modifications, as described by Donata in 2002 [26]. DPPH solution was prepared in ethanol and sample was dissolved in DMSO, then 95 μL of DPPH solution and 5 μL of sample were taken in a micro liter plate. The final well concentration for DPPH was maintained at 300 μM and for sample at 500 μg/mL. Reaction mixture was incubated at 37 °C for 30 min, and the final absorbance was recorded on microplate reader at 515 nm. Radical quenching efficacy of compounds was determined by comparison with DMSO treated blank (control).

Radical Scavenging Activity (% RSA) of extracts was calculated by comparing it with a control.

$$\%RSA = 100 - \{(As/Ac) \times 100\}$$

As = Absorbance of sample, Ac = Absorbance of control

2.7. Superoxide anion scavenging assay

This protocol was exploited for the determination of superoxide anion scavenging ability of test compounds by using NADH/PMS system with certain changes, earlier described by Freda in 2003 [27]. Method of superoxide scavenging assay was carried out in a 96-well micro liter plate. Each reaction well contains 10 μL of extract (500 μg/mL), 40 μL of NADH (100 μM), 40 μL of NBT, 20 μL of PMS and 90 μL of phosphate buffer (100 μM, pH 7.4). Buffer was used for the preparation of reagents, whereas DMSO is used for test compound. The experiment was carried out at room temperature and generation of superoxide was monitored by employing spectrophotometer at 560 nm. A control sample was set in parallel for the comparison with the test sample, since the decrease in absorbance of test sample signifies its antioxidant activity.

Radical Scavenging Activity (% RSA) of extracts was calculated by comparison with a control.

$$\%RSA = 100 - \{(As/Ac) \times 100\}$$

As = Absorbance of sample, Ac = Absorbance of control