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Constituents from fruits of *Cupressus sempervirens*☆

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ABSTRACT

Two new phenolic glycosides (**1**, **2**), along with fourteen known compounds (**3**–**16**) have been isolated from the fruit of *Cupressus sempervirens*. The structures of these compounds were determined by spectroscopic analysis and were evaluated for their inhibitory activity against glycogen phosphorylase and glucose-6-phosphatase enzymes. Compounds **14** showed a moderate inhibition against glucose-6-phosphatase and **15** against glycogen phosphorylase enzymes.

1. Introduction

The genus *Cupressus* (cupressaceae), comprising twelve species, is distributed in North America, the Mediterranean region and subtropical Asia at high altitudes. Five of them were reported as part of the Indian flora viz *Cupressus torulosa*, *C. sempervirens, C. funebris, C. lusitanica (C. glauca)* and *C. macrocarpa*, while *C. cashmeriana* has been considered to be only a form of *C. torulosa* [1]. Phytopreparation obtained from the core and young branches of *C. sempervirens* were reported to have antiseptic, aromatherapeutic, astringent, balsamic and anti-inflammatory activities [2]. Cypress is also described to exert antispasmodic, astringent, antiseptic, deodorant, and diuretic effects, to promote venous circulation to the kidneys and bladder area, and finally to improve bladder tone and as a coadiuvant in therapy of urinary incontinence and enuresis [3–9].

A proanthocyanidin fraction (MW 1500–2000 Da) from *C. sempervirens* exhibited a valuable antiviral activity in vitro against two retroviruses, HIV and HTLV III B. In comparison with AZT no toxicity was observed with PPC at concentration of 50 μg/mL, which exceeded the IC_{50} values (1.5 to 15 μg/mL for HIV and 5 to 25 μg/mL for HTLV) [10]. The MeOH extract of leaves of this plant was shown to exert hepatoprotective activity against normal and CCl (4)-rats. The MeOH extract also exhibited free radical scavenging activity against stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) as well as some of the isolated phenolic compounds (e.g. quercetin, rutin, caffeic acid, and *p*-coumaric acid) in comparison with <alpha>-tocopherol and butylated hydroxyl toluene (BHT) as standard antioxidants using ESR technique [11]. Essential oil of this plant exhibited antimicrobial [12,13] and antifungal activities [14]. Several monoterpenes, diterpenes and flavonoid glycoside and biflavonoids were isolated from this plant [15–17].

As a part of our ongoing studies aimed to phytochemically and pharmacologically characterize the title plant, we found that EtOH extract of fruit of *C. sempervirens* shows significant inhibitory activity against glucose-6-phosphatase enzyme. Therefore, we decided to carry out a detailed study aimed to investigate the chemical composition of *C. sempervirens*. In particular in the present paper we wish to report the isolation and characterisation of two new phenolic glycosides (**1**, **2**) along with fourteen known compounds (**3**–**16**) with their

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glycogen phosphorylase and glucose-6-phosphatase inhibitory activities. The known compounds were identified by using spectroscopic methods including, mass, 1D and 2D NMR analysis and also by comparison data already reported in the literature.

n-Butanol fraction yielded two new phenolic glycosides (**1**, **2**), and five known compounds: catechin (**3**) [18] epicatechin (**4**) [19], neolignans (**5**) [20], 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-rhamnopyranoxypropyl)-2-hydroxyphenoxy] 1,3-propanediol) (**6**) and (1-(4-hydroxyphenyl)-2-[4-(3-glucopyranoxypropyl)-2-methoxyphenoxy]-1,3-propanediol) (**7**) [21]. Chloroform fraction yielded nine known compounds sugiol (**8**) [22], communic acid (**9**) [23], junepediol (**10**) [24], sandracopimaric acid (**11**) [25] enantio olevaric acid (**12**) [26] imbricatolic acid (**13**) [27], acetoxyimbricatolic acid (**14**) [28], ferruginol (**15**) [29] and abita-8, 11, 13-triene-20-ol (**16**) [30]. Among these, compounds **3**–**7** and **10** have been reported from the title plant for the fist time.

2. Experimental

2.1. General procedures

Optical rotations were measured on a Perkin-Elmer model 241 digital polarimeter. UV spectra were obtained on a Perkin-Elmer λ-15 UV spectrophotometer. IR spectra were recorded on a Perkin-Elmer RX-1 spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 300 MHz NMR spectrometer. ESMS on an Advantage Max LCQ Thermo-Finnigan mass spectrometer and FAB-MS were carried out on a JEOL SX 102/DA-6000 mass spectrometer. CC was performed using silica gel (230–400 mesh). TLC was carried out on precoated silica gel plates 60 F254 or RP-18 F254 plates (Merck). Spots were visualized by UV light or by spraying with H_2SO_4–MeOH or anisaldehyde–H_2SO_4 and vanillin-H_2SO_4 reagents.

2.2. Plant material

C. *sempervirens* leaves were collected from Almora, Uttrakhand India, in January, 2007. The collection and authentication was done by Dr. Kamal R. Arya, Botany Division, Central Drug Research Institute. The voucher specimen (No. 24421) is stored in the herbarium of the institute.

2.3. Extraction and isolation

The dried and powdered fruit (8.00 kg) of C. *sempervirens* was percolated three times successively with 95% ethanol at room temperature. The combined extract was filtered and concentrated under reduced pressure at 40 °C afforded dark brown residue (900 g). Ethanol extract (800 g) was triturated with hexane (200 g) and the hexane insoluble portion was dissolved in water, which was successively extracted with chloroform (5 L×5) and *n*-butanol (5 L×5) and yielded fractions of chloroform (120 g), *n*-butanol (300 g) and water (180 g).

The butanol fraction was subjected to column chromatography on silica gel (60–120 mesh) and eluted with a gradient of $CHCl_3$–MeOH (95:05) to MeOH–H_2O (95:05), fifty fractions were collected (500 ml each) and their composition was monitored by TLC, those fractions showing similar TLC profile grouped into ten major fractions (1–10). Compound **1** was obtained as an amorphous powder from the sub-fraction 2. Sub-fraction 3, chromatographed over Resin HP-20 using gradient of H_2O–MeOH, yielded fraction 11–15. Fraction 13 purified by combination of Sephadex G-25 and RP-18 column chromatography (H_2O–MeOH), afforded compounds **3** and **4**. Compound **5** was purified from sub-fraction 12, by RP-18, followed by silica gel. Sub-fraction 14 was re-chromatographed over RP-18 (H_2O–MeOH) and followed by silica gel (MeOH–$CHCl_3$ 5:95) in isocratic manner to give compounds **1**, **6** and **7**.

Chloroform fraction was subject to column chromatography (CC) over silica gel (100–200 mesh) and eluted with gradient systems of increasing polarity hexane: EtOAc (0–100%) and finally MeOH. 50 Fractions were collected and combined on the basis of their TLC profiles to afford 19 fractions 1–19. Fraction 1 was further purified on silica gel (230–400 mesh) with hexane–ethyl acetate (98:02) elute, yielded compound **8**, which was crystallized in chloroform–hexane mixture as colourless needles (150 mg). Fraction 2, containing the mixture of two compounds, was also chromatographed over silica gel (230–400 mesh) eluted with hexane: ethyl acetate (95:5) in isocratic manner, afforded two compounds **9** (25 mg) and **11** (20 mg). Fraction 4 was subjected to silica gel CC (230–400 mesh), on elution with hexane:ethyl acetate (10:0–0:10) mixture, obtained sub-fraction F1–F10. Sub-fraction F2 was purified by repeated CC over silica gel (230–400 mesh) (hexane:ethyl acetate) yielded compounds **16** (11 mg) and **14** (15 mg). In the same way, sub-fraction F3 afforded compounds **15** (17 mg) and **13** (17 mg). Where as sub-fraction F4 delivered compounds **10** (10 mg) and **12** on silica gel (230–400 mesh) column chromatography employing hexane:ethyl acetate (1:1) as eluents.

Table 1
^{1}H and ^{13}C NMR data of compound **1** in DMSO-d_6.

Position	d_H (*J* in Hz)	d_C
2		157.7
3		134.6
4		178.1
5		161.9
6	6.19 (brs)	99.1
7		164.7
8	6.38(brs)	94.1
9		156.9
10		104.5
1′		121.1
2′	7.26 (d, 2.3)	115.9
3′		145.6
4′		148.9
5′	6.84 (d, 8.1)	116.0
6′	7.22 (dd, 8.1, 2.3)	121.6
1″	5.22 (d, 1.2)	102.2
2″	3.90 (m)	70.7
3″	3.14 (m)	71.6
4″	3.40 (m)	71.0
5″	3.15 (m)	70.5
6″	0.78 (d, 5.5)	17.4
OCH_3	3.14 (s)	49.0