SHORT REPORT

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Chemical Constituents and Antioxidant Activity of *Geranium wallichianum*

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Abstract: The study of the chemical constituents of the whole plant of *Geranium wallichianum* (Geraniaceae) has resulted in the isolation and characterization of six compounds. These six compounds were identified as ursolic acid (**1**), *β*-sitosterol (**2**), stigmasterol (**3**), *β*-sitosterol galactoside (**4**), herniarin (**5**), and 2,4,6-trihydroxyethylbenzoate (**6**) which were isolated for the first time from *Geranium wallichianum.* The above compounds were individually identified by spectroscopic analyses and comparisons with reported data. The antioxidant potential of *Geranium wallichianum* extracts has been investigated by DPPH radical scavenging assay and EtOAc extract was found to be most potent with IC_{50} 19.05 ug/mL.

Keywords: *Geranium wallichianum*; antioxidant activity; DPPH, coumarin; triterpenoid; steroid.

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1. Plant Source

Geranium wallichianum belongs to the family Geraniaceae. It is a tall much branched procumbent or erect perennial herb found in the Himalayas, from Kashmir to Nepal at the altitude of 7000-11000 feet [1]. Also found in Chitral, Dir, Swat, Hazara, Muree hills, Pounch, and Kashmir [1]. The herb evidently possesses the astringent properties of the genus to a marked degree. The rootstock is used as a substitute for that of Cotis teeta Wall in eyes troubles. The herb is also used in the treatment of toothache. Here an attempt has been made to study chemical constituents and antioxidant activity of *G. wallichianum* extracts by DPPH radical scavenging assay [1].

The whole plant *G. wallichianum* was collected from northern area of Pakistan, in July 1999, and identified by Dr. Jahandar Shah (plant taxonomist) at the Department of Botany, University of Peshawar, Pakistan. A voucher specimen (No. ISP 203) has been deposited at the herbarium of the Botany Department University of Peshawar, Pakistan.

2. Previous Studies

There are no reports on phytochemical investigation and radical scavenging properties of *G. wallichianum*.

3. Present Study

The air-dried underground plant part (6 kg) of *G. wallichianum* was exhaustively extracted with methanol at room temperature (10 days). The extract was evaporated to dryness yielding 130.5 g residue. The 125 g of the residue was fractionated with *n*-hexane, chloroform, EtOAc and *n*-butanol. The EtOAc extract (32 g) was subjected to a silica gel column chromatography packed using different solvent systems and yielded five fractions (A-E). Compound **1** was obtained from fraction A after subjecting to CC eluted with *n*-hexane:$CHCl_3$ (7:3) as colorless needles (30 mg). Similarly fraction B was subjected to CC using solvent system *n*-hexane:$CHCl_3$ (8:2) and compound **3** (35.8 mg) was obtained as an amorphous solid. The fraction C was subjected to silica gel column chromatography to obtain amorphous solid (35 mg) of compounds **4** and **5** (20 mg) were obtained from fraction D after subjected to repeated chromatographic purifications using *n*-hexane:EtOAc (3:8). Finally compound **6** (55 mg) was obtained from fraction E eluted with *n*-hexane:$CHCl_3$ (7:3).

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay: The antioxidant activity of petrol ether, $CHCl_3$, EtOAc, *n*-butanol and aqueous extract of *G. wallichianum* were assessed on the basis of radical scavenging effect of stable DPPH free radicals [2]. The reaction mixture containing 5 µL of test sample (200 µg/mL in DMSO) and 95 µL of DPPH (sigma, 300 µM in ethanol) was then taken in 96-well microtiter plate (Molecular Devices, USA) and incubated in Elisa at 37ºC for 30 min., the absorbance was measured at 515 nm. Percent radical scavenging activity was determined by comparison with a DMSO treated control.

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Results and Discussion: The whole plant extract of *G. wallichianum* was fractionated by silica gel column chromatography to give several fractions, which were further chromatographed on silica gel to afford one triterpene (**1**), three steroids (**2**-**4**), one coumarin (**5**), and one benzoic acid derivative (Figure 1). The compounds were identified as ursolic acid (**1**) [3], stigmasterol (**2**) [4], *β*-sitosterol (**3**) [4], *β*-sitosterol galactoside (**4**) [5], herniarin (**5**) [6], and 2,4,6-trihydroxyethylbenzoate (**6**) [7] by comparison of their 1D and 2D NMR spectral data with the reported data in the literature.

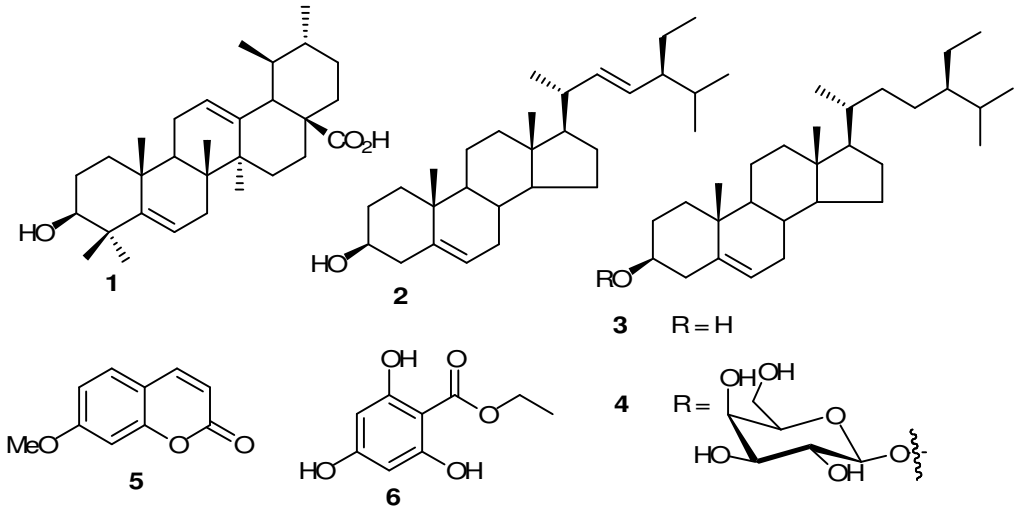

Figure 1. Compounds **1–6** isolated from *G. wallichianum*.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity. In the past decade natural antioxidants have generated considerable attention in preventive medicine. Consequently, much attention has been directed toward the discovery of new natural antioxidants, including herbal products, aimed at quenching biologically harmful radicals. The numerous pathological events are associated with generation of reactive oxygen species (ROS) constituting a key mechanism of tissue injury and they are significant relevance in the inflammation process, in risk of cardiovascular disease [8,9] and in pathology of arteriosclerosis, malaria and rheumatoid arthritis and could play a role in neurodegenerative disease and premature aging [10-12]. The evidence for a role of ROS in human disease makes the search of new antioxidants especially relevant. Free radicals play an important role in carcinogenesis through their involvement in breaking of DNA strand [13].

Figure 2 represents the radical scavenging activity of the extracts from *G. wallichianum.* The antioxidant activity of *G. wallichianum* extracts was tested by measuring their capacity to scavenge DPPH radical. The EtOAc extract showed the highest antioxidant activity with an IC_{50} value of 19.05±0.90 µg /mL, while *n*-butanol extract (IC_{50} 24.133 ±0.56) and aqueous extract (IC_{50} 25.35±1.20) showed less activity, and the standard antioxidant 3-*t*-butyl-4-hydroxyanisole showed an IC_{50} value of 8.0725±0.65. The tested samples reduced the stable radical DPPH to the yellow-colored diphenyl-picrylhydrazine. DPPH radicals have been widely used to evaluate the antioxidant properties of natural products as well as plant extracts [14].

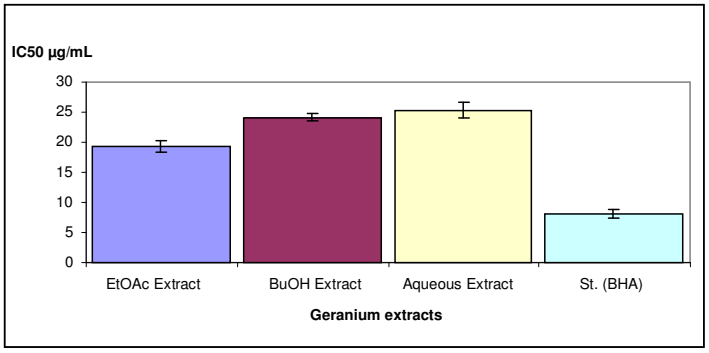

Figure 2. DPPH radical scavenging capacity of ethyl acetate, butanol and aqueous extracts of *Geranium wallichianum* and standard (BHA). Values are expressed as IC_{50} µg/mL ± S.E.M of DPPH. Each point represents the standard error of mean of three determinations.

Conclusion. In this work, we found that all tested materials were able to quench this radical in a concentration-dependent manner. In the DPPH assay the EtOAc extract was found to be most potent with IC_{50} 19.05 ug/mL than *n*-butanol and aqueous extract, thus indicating a possible synergistic interaction of the constituents or the presence of more potent antioxidants.

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