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SECONDARY METABOLITES FROM *Artemisia parviflora* AND *Convolvulus pseudocantabrica* OF PAKISTANI ORIGIN

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Man has used plants since the dawn of civilization to treat various diseases and disorders. This interaction of man and plants led to the establishment of the traditional knowledge of plants. This traditional knowledge of ethnic groups around the globe forms the basis of modern plant-based industry.

Artemisia is a large, diverse, and economically important genus of the family Asteraceae. It comprises more than 500 species throughout the world [1]. There are about 38 species found in Pakistan, out of which 12 species are popularly used by Pakistanis as food, ornaments, fumigants, and medicines [1]. *Artemisia parviflora* is locally known as "Kharkalich" [2] and distributed in the north and northwest regions of Pakistan [3]. The chemical composition of the essential oil from aerial parts of *A. parviflora* has also been reported [4]. It was found to possess antiviral activity [4]. These parts of the plant are dried and used in ground form for stomachache, high blood pressure, and diabetes. It is also effective as an anthelmintic [5]. The methanolic extract of the leaves was found to possess higher toxicity against *Anopheles stephensi* [6].

The genus *Convolvulus* belongs to the family Convolvulaceae. *Convolvulus pseudocantabrica* is distributed mainly in temperate (i.e., Afghanistan and Iran) and tropical regions of Asia (i.e., Pakistan). Investigations have shown that the genus *Convolvulus* is a rich source of tropane alkaloids [7]. Convolvine, a tropane alkaloid isolated from *C. pseudocantabrica* [7], has shown the characteristics of a sedative and nootropic agent [8].

According to the reported literature, no phytochemical investigations have been carried out on *Artemisia parviflora* and *Convolvulus pseudocantabrica*. We report here the isolation and structure elucidation of three known compounds, α-amyrin (**1**), β-amyrin (**2**), and scopoletin (**3**) from *Artemisia parviflora* and lupeol (**4**) from *Convovulus pseudocantabrica* Schrenk, which were isolated for the first time from these plants. The structures of these compounds were deduced by comparison of their spectral data with those reported in the literature.

The air-dried plant of *Artemisia parviflora* (1.8 kg) was extracted with methanol (5 L) at room temperature (30°C) for 15 days. After evaporation of the solvent, a crude extract (356.2 g) was obtained, which was dissolved in distilled water (500 mL) and defatted with petroleum ether (2.5 L). The defatted aqueous layer was extracted with $CHCl_3$ (3 L) to afford a chloroform fraction (42.6 g). The remaining aqueous portion was extracted with ethyl acetate (3 L) to obtain an ethyl acetate fraction (28.4 g).

The resulting chloroform extract (42.6 g) was subjected to column chromatography (CC) on a silica gel column (70–230 mesh, 400 g), and the column was eluted with about 5 liters of petroleum ether: $CHCl_3$ to $CHCl_3$: MeOH mixtures with increasing polarity (from 9:1 to 8:2, respectively) to afford ten major fractions (APC-1 to APC-10). Compounds **1** (16.4 mg, petroleum ether–$CHCl_3$, 6.8:3.2), **2** (14.8 mg, petroleum ether–$CHCl_3$, 6.9:3.1), and **3** (56.8 mg, petroleum ether–$CHCl_3$, 2.5:7.5) were isolated from these column fractions by repeated column (CC) and preparative thin-layer (TLC) chromatographic techniques.

The air-dried plant of *Convovulus pseudocantabrica* (800 g) was extracted with methanol (3 L) at room temperature (30°C) for 15 days. After evaporation of the solvent, a crude extract (142.6 g) was obtained, which was dissolved in distilled water (300 mL) and defatted with petroleum ether (2.5 L). The defatted aqueous layer was extracted with $CHCl_3$ (3 L) to afford a chloroform fraction (28.4 g).

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The resulting chloroform extract (28.4 g) was subjected to column chromatography (CC) on a silica gel column (70–230 mesh, 250 g), and the column was eluted with about 3 liters of petroleum ether–$CHCl_3$ to $CHCl_3$–MeOH mixtures with increasing polarity (from 9:1 to 8:2, respectively) to afford six major fractions (CPC-1 to CPC-6). Compound **4** (26.5 mg, petroleum ether–$CHCl_3$, 7.2:2.8) was isolated from these column fractions by repeated column (CC) chromatographic techniques.

The present study on the methanolic extracts of *Artemisia parviflora* and *Convovulus pseudocanabrica* Schrenk of Pakistani origin has resulted in the isolation and characterization of three pentacyclic triterpenoids, **1**, **2**, and **4**, and a coumarin, **3**. The structures of these compounds were identified on the basis of spectroscopic methods and by comparison with reported literature. They were found to be α-amyrin (**1**) [9], β-amyrin (**2**) [9], and scopoletin (**3**) [10] from *Artemisia parviflora*, and lupeol (**4**) [11] from *Convovulus pseudocanabrica* Schrenk. These compounds were isolated for the first time from these plants.

The ^1H NMR spectra were recorded in $CDCl_3$ on Bruker AM-400 and AMX-500 NMR spectrometers with TMS as an internal standard using the UNIX operating system at 400 and 500 MHz, respectively. The ^{13}C NMR spectra were recorded in $CDCl_3$ at 125 MHz on a Bruker AMX-500 NMR spectrometer. The EI-MS spectra were recorded on a double- focusing mass spectrometer (Varian MAT 311 A). HR-EI-MS were recorded on Jeol JMS 600 and HX 110 mass spectrometers with the data system DA 5000. The IR spectra were recorded on a Jasco A-302 spectrophotometer. The UV spectra were recorded in methanol on a Hitachi UV 3200 spectrophotometer. The optical rotations were measured in methanol on a JASCO DIP-360 digital polarimeter. Melting points were recorded on a Yanaco MP-S3 micro-melting point apparatus and are uncorrected. Column chromatography (CC) was carried out on a silica gel column (70–230 mesh). Purity of the samples was checked by TLC on pre-coated silica gel GF-254 preparative plates (20 × 20 cm, 0.25 mm thick, Merck) and were detected under UV light (254 and 366 nm).

The aerial parts of *Artemisia parviflora* (2.5 kg) and *Convolvulus pseudocantabrica* (1.2 kg) were collected in July, 2001 from Booni valley of District Chitral (Pakistan), and were dried in the air. The plants were identified by Mr. Tahir Ali, plant taxonomist, Department of Botany, University of Karachi, Karachi, Pakistan. Herbarium specimens KUH # 94321 and KUH # 83724 of *Artemisia parviflora* and *Convolvulus pseudocantabrica*, respectively, were deposited as a reference at the Department of Botany, University of Karachi, Karachi, Pakistan.

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