Chemical Compounds Isolated from Aerial Part of *Primula auriculata* L.

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Abstract

Aerial parts of *Primula* genus (*Primulaceae*) are well known to treat various ailments in Iranian Traditional Medicine. Our preliminary studies demonstrated that the medium polar to polar extracts of *Primula auriculata* had a significant cytotoxic activity and Induction of apoptosis effects on some cancerous cell lines. The current study focused on phytochemical investigation and characterization the compounds from *P. auriculata* active extracts. Dried and powdered aerial part of plant was macerated with petroleum ether, dichloromethane, and methanol successively, for 24 hours, three times. Dichloromethane and methanolic extracts were separately examined by different chromatographic techniques such as VLC, CC, and TLC to Fractionation and isolation compounds present in the plant extracts. The structures of purified compounds were elucidated on the basis of spectral data, particularly H-NMR and C-NMR experiments. 2-phenylchromone and 3, 4, 5-trihydroxy benzoic acid was identified for the first time from *P. auriculata*. In this research we have isolated compounds from pharmacological active extracts which is most likely responsible for cytotoxic action of *P. auriculata*. Further studies are required to determine activity and mechanism of action of isolated compounds.

Keywords: *Primula auriculata*, Primulaceae, flavonoid, gallic acid, plant extract, Isolation and structural elucidation

1. Introduction

*Primula* is a large genus in Primulaceae (Fam) which comprised of 425 species in the world. It is represented by 6 species which is spread across some parts of north (Alborz, Gachsar, Kandovan) and west cold district of Iran as well as Anatoly, Iraq, Pakistan, Mongolia, Caucasus and central Asia [1]. The glands of *Primula* species produce farinas (formed as rod
or needle-like crystals) and/or exudates (sticky secretions) [2]. Various *Primula* species have been used traditionally in the treatment of epilepsy, insomnia, asthma and food poisoning [3]. They have revealed biological and pharmacological effects such as antimicrobial, antioxidant, anxiolytic, antiviral, expectorant, antidiabetic, antileishmania, and cytotoxicity [4-6].

*Primula auriculata* known under the Persian name “Pamchal e juybari” is a perennial, spring herb which is grows in wet mountain meadows. In Iranian ethno medicine, white powder from plant inflorescence (locally named Tootia), is reported to be useful in prevention and treatment of eye infection, Trachomatis and glaucoma. In previous studies, the methanolic extract from aerial part of this species was examined by MTT assay for cytotoxic activity on various human cancer cell lines and a normal cell line. The results showed significant effects on (MCF7, human breast adenocarcinoma, HepG2, hepatocellular carcinoma and HT-29, human colon carcinoma) with IC50 of 25.79, 35.79 and 43.34 μg.mL−1, respectively and no cytotoxicity against normal cells [7]. Therefore, by using different solvents (petroleum ether, dichloreomethan (CH2Cl2) and methanol (MeOH)), three extracts with a range of polarity were prepared and screened for cytotoxic and apoptosis induction effects. CH2Cl2 and MeOH extract decreased cell viability in HT-29 cells (IC50 of 33.20 and 58.05 μg.mL−1). This toxicity was consistent with morphologic changes including reduction in cell volume and rounding. Using Acridine orange (AO)-ethidium bromide (EB) double staining cell morphological analysis, Annexin V-propidium iodide staining, TUNEL and Caspase-3 activity assay), it was revealed that apoptosis is involved in *P.auriculata* induced HT-29 cell death [8]. Previous phytochemical investigation of *Primula* species, have reported that this genus is an abundant source of flavonoids (aglycone and glycosylated), phenolic, and triterpenoid saponins [9-11]. These compounds have many pharmacological and biological activities including antioxidantive, anti-inflammatory, and anti-tumor. As a part of our ongoing endeavor to find compounds from *Primula auriculata* bioactive extracts, the present study is about the isolation and structure elucidation of phytochemicals from CH2Cl2 and MeOH extracts of plant for the first time.

2. Material and Methods

2.1. General Experimental Procedures

The NMR spectra were recorded on a Bruker Avance AV-300 (1H) and AV-100 (13C), using CDCl3 and DMSO-d6 as solvent. EI-MS spectra were measured in a 7890 GC/7200 Q-TOF MS system. TLC was carried out on pre-coated aluminum foil silica gel 60 F254 (Merck Co, Germany). Column chromatography (CC) was performed with silica gel 60 (Merck Co, Germany) and Sephadex LH-20 (Pharmacia Inc., Piscataway, NJ, USA). For vacuum liquid chromatography (VLC), silica gel 60 G (15 μm, Merck Co, Germany) and were applied. Thin layer chromatography detection was achieved by spraying the silica gel plates with Anisaldehyde-sulphuric acid reagent (AS), Vanillin-sulphuric acid reagent (VS), Natural products-polyethylene
2.2. Plant Material

Plant materials were collected from Alvand (Hamedan province, west Iran) in June 2013. A voucher specimen 3224 (TMRC) was deposited at the herbarium of Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.3. Extraction and Isolation

About 1 kg of the air-dried powdered aerial part of plant was extracted with petroleum ether, CH₂Cl₂ and MeOH respectively, at room temperature (2.5 L x 72 h x 3 days). The extracts were concentrated under reduced pressure then the following residues were obtained: R₀ (10g) R₀ (4 g) and R₅ (16 g). R₀ was subjected to VLC (65 x 15 cm, SiO₂, 50g) with gradient elution using solvents of increasing polarity, petroleum ether and ethyl acetate. Totally 6 fractions (Fr. A- Fr. F) were obtained according to TLC analyses. The spots were visualized by spraying with Anisaldehyde-sulphuric acid reagent (AS), Vanillin-sulphuric acid reagent (VS) and Natural products-polyethylene glycol reagent (NP/PEG). Fr.E separately chromatographed on a Sephadex LH-20 column (3x90 cm, 50g) using MeOH: H₂O as eluent to obtain 20 fractions which were combined together in to 4 final fractions. Fr.E2 (14mg) afforded compound 1 (Figure 1). R₅ was redissolved in MeOH: H₂O and partitioned in a separating funnel with ethyl acetate and methanolic part was subjected on a Sephadex LH-20 column (2x30 cm, 35g) eluted with MeOH:H₂O to yield 4 final fractions (M1-M4) by TLC monitoring. Compound 2 (11mg) was given from M3 (Figure 2).

2.4. Spectroscopic Properties of Compounds

Compound 1, C₁₃H₁₀O₂, white Chrystal, 14mg : ¹H NMR (300 MHz, CDCl₃, δ, ppm, J/Hz): 8.26 (1H, d, J = 8.1 Hz, H-5), 6.87 (1H, s, H-3), 7.45 (1H, m, H-6), 7.73 (1H, m, H-7), 7.59 (1H, m, H-8), 7.96 (2H, m, H-2’,6’), 7.57 (3H, m, H-3’,5’,4’). ¹³C NMR (CDCl₃, 100 MHz, δ, ppm): 178.5 (C-4), 163.4 (C-2), 131.6 (C-4’), 118.1 (C-8), 156.3 (C-9), 125.2 (C-6), 133.8 (C-7), 126.3 (C-2’,6’), 131.8 (C-1’), 129 (C-3’,5’), 125.6 (C-5), 107.6 (C-3), 124 (C-10). EIMS m/z: 222 [M]+[12].

Compound 2, C₃H₅O₃, white/yellow chrystal, 12mg : ¹H NMR (300 MHz, DMSO-d₆, δ, ppm, J/H): 6.91 (4H, s, H-2,6), 12.23 (1H, s, H-COOH), ¹³C NMR (CDCl₃, 100 MHz, δ, ppm): 167.9 (C-7), 145.8 (C-3), 138.4 (C-5), 120.9 (C-1,4), 109.1 (C-2,6), EIMS m/z: 169.9 [M⁺][13].

3. Results and Discussion

The structures of the isolated compounds were elucidated by spectroscopic methods (MS, ¹H NMR and ¹³C NMR) and by comparison with those reported literature data. They were elucidated as 2-phenylchromone (1) and 3, 4, 5-trihydroxy benzoic acid (2) (Figure 3, 4). The compounds were identified for the first time from Primula auriculata.

Its ¹³C NMR spectrum of compound 1 showed 15 carbon signals sorted in to 10 CH, 4 quaternary C and a C=O group. The ¹H NMR spectrum revealed a set of six proton signals at δH 7.45- 8.26 corresponding to the aromatic hydrogen in A and B ring, besides a singlet at δH 6.87, which was integrated with one proton is...
typical for H-3. In the MS, two prominent fragments, retro-Diels–Alder (RDA) ions, at m/z 102 and 120 due to [A1]⁺ and [B1]⁺ were observed besides a molecular ion at m/z 222 (C₁₅H₁₀O₂), which indicated that 1 was flavone skeleton with unsubstituted pattern. The
Natural compounds from *Primula auriculata* L.

Fragment [M-28] is indicated C=O group and loose of the unit 92 m/z is assigned to tropylium ion. On this basis compound 1 was characterized as 2-phenylchromone (flavone).

The $^{13}$C NMR spectrum of compound 2 showed 7 signals sorted in to 2 CH, 4 quaternary C and a COOH group. In the $^1$HNMR spectrum, one four-proton singlet at 6.91 assigned to H-2, 6 was observed. Also, a weak singlet at 12.23 was assigned to carboxylic moiety. The MS showed a parent peak at $m/z$ 169.9, an empirical formula of which corresponds to C$_7$H$_6$O$_5$. Thus compound 2 was identified as 3, 4, 5-trihydroxy benzoic acid (gallic acid).

Aerial part of Primulaceae plant is coated with powdery, waxy, and sometimes oily farinose. The irregular substitution flavonoids are common and usually the main component of their exudate and aerial part flavonoid composition. The uniqueness of occurrence of Primula type flavones are thought to be useful chemotaxonomic markers as well as uv-protectant agent for Primula genus [14]. Flavone itself is one of these irregular skeletons and rare types of component in natural products. Flavone
with lacking any O-substitution has been occurred to date only in certain species in the Primulaceae. It seems that glandular cell in these species have their own specific flavonoid biosynthetic pathway. In previous studies, unsubstitution flavone was isolated from aerial part of several another Primula species such as P.albenis, P.veris, and P.pulverulenta and some limited species in Dionysia genus [2,15, 16]. To our knowledge, very limited biological effects have been reported from this compound. Crude extract, different fractions of P. macrophylla, and 2-phenylchromone (flavone) as a pure compound which was isolated from chloroform fraction exhibited significant cytotoxicity and antimicrobial activities, while cytotoxicity in brine shrimp test and anti-leishmanial activities for flavone were reported as (LD_{50} = 2.0116 µg/ml) and (IC_{50} = 25 µg/ml), respectively[6]. In another study, the methanolic extract of P. macrophylla have been investigated for cytotoxicity by MTT test against human colon cancer cell line (Colo-205) along with evaluating the effects on apoptosis induction, cell cycle arrest and mitochondrial membrane potential. Results indicated that the extract was so effective and decrease cell viability in dose dependent manner (IC_{50} = 26.17 µg/ml). It was induced apoptosis due to DNA fragmentation and through a mitochondrial pathway in Colo-205 cells [17].

3, 4, 5-tri hydroxyl benzoic acid (gallic acid) is a natural compound isolated from different plants. It is a type of phenolic acid, found both free and as part of hydrolyzable tannins. The cytotoxic effect and induction of apoptosis of gallic acid was investigated in various cancer cells (leukemia, lung, and stomach) especially in several colonic cancer cells [18]. It also exhibits significant antioxidant and anti-inflammatory activities [19].

4. Conclusion

Consequently, we have identified two phenolic compounds isolated from Primula auriculata as cytotoxic agents. This research supports the antiproliferative effect of P. auriculata especially in colonic cancer cell lines. Future studies will be directed to investigate other active compounds.

References

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