



***Campanula latifolia*, Giant Bellflower; Ethno-botany, Phytochemical and Antioxidant Evaluation**

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Abstract

Campanula latifolia is a plant of Campanulaceae family, commonly known as Giant bellflower. *Campanula* genus is mentioned in Iranian medicine sources with various names such as kaff-e-Maryam and Gol-e-estekani. The aim of this study was to assay the different groups of chemical compounds and to investigate the antioxidant effects of ethanolic extract of aerial parts and root of this plant. After providing 70% alcoholic extract of aerial parts and root, qualitative tests were performed to evaluate anthocyanins, saponins, cardiac glycosides, flavonoids, tannins, sterols, triterpenoids, and alkaloids. Tests of antioxidant effects were evaluated by DPPH free radical scavenging effect and the content of phenolic compounds in the extracts using Folin-Ciocalteu method. Phytochemical studies indicate that there are compounds of cardiac glycoside, triterpene, and sterols. In the quantitative analysis of the compounds of the two plant extracts, the total amount of phenols were measured 0.053 and 0.037 (mg Gallic acid/g extract) in aerial parts and root extracts, respectively. The amount of IC₅₀ of DPPH radical scavenging activity of the plant has also been 1163.636 µg / ml for the aerial parts extract and 1419.354 µg / ml for the root extract. Considering the results and the presence of cardiac glycosides, triterpenoids, and sterols, further phytochemical and pharmacological studies are suggested.

Keywords: *Campanula latifolia*, Phytochemical evaluation, Antioxidant, DPPH, Folin Ciocalteu

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Introduction

Campanula latifolia is from the Campanulaceae family. The genus *Campanula* includes about 420 species distributed throughout the Arctic and northern temperate regions, most abundant from the Mediterranean to the Caucasus. Plants of this genus are generally endemic in the Mediterranean areas, meadows and mountain hills. All species are herbaceous, and their name refers to the bell-shaped blue flowers in most of these species [1]. *Campanula* in Iran has 44 species distributed in all northern parts of the country from Khorasan to West Azerbaijan and throughout the Zagros belt to Bandar Abbas and central areas such as Kerman; about 14 of them are endemics [2]. Among the plants of this genus, annual, biennial and evergreen species can be seen, varying from 5 cm to two meters high. The flowers in this genus are single clusters, and the calyx has five lobes from 2 to 5 cm in size. The color of the petals is primarily blue to violet, but sometimes there are colors like white or pink. The fruit also contains a lot of seeds in the capsule [3,4]. Roots of *Campanula* contain stimulant compounds and the leaves were used to treat warts [5].

Campanula plants are listed in Iranian medicine sources with various names, including: kaffe- Maryam, Gol-e-estekani, midion, dhanab ol oyal, jaras kanterburi, zahjaras, asnab, and reyam [6].

Several species including *Campanula glomerata*, *C. persicifolia*, *C. rotundifolia*, *C. bononiensis*, *C. sibirica* and *C. patula* have been used locally for the preparation of traditional drugs in Russian folk medicine, and in Italy.

C. medium, *C. cervicaria*, *C. rotundifolia*, *C. latifolia* and *C. trachelium* have similar uses. They were used to treat epilepsy, neurological diseases, coughing, headache, rheumatism, and inflammation. Many of the medicinal properties of these species are similar to the use of *Platycodon grandiflorum*, which was used for the manufacture of medication in the Eastern medicine, and was originally classified in *Campanula* genus [7].

The roots and leaves of *C. rapunculoides* can be used as vegetables. Some species have also been studied to identify the content of secondary metabolites, and alkaloid compounds such as campedin and (-) lobeline have been identified from *C. medium*. *Campanula latifolia* are potentially unused plants that can be as a source of secondary medicinal metabolites [7].

Few studies have been reported on the pharmacological effects of *Campanula*. In a study by Yildirim et al. [8], the antibacterial and antitumor effects of some plants collected from Turkey, including two species of *Campanula glomerata* L. and *Campanula olympica* Boiss, have been reported [8].

Another study in 2013 examined the chemical compounds, and antimicrobial effects of the aqueous extract and essential oil of *Campanula portensclagiana* collected from Croatia. Based on the results of this study, the compounds of diterpene alcohol with the labda-13(16), 14-dien-8-ol structures were the main components of this plant. The volatile compounds separated from this plant have shown strong antimicrobial effects, especially against gram-negative pathogens [9]. The antinociceptive effects of

Campanula punctate hydro-alcoholic extract in different animal models are likely to be mediated by 2α -adrenergic receptors [10]. The purpose of this study was to investigate the use of *Campanula* species in other countries, to investigate the presence of phytochemical compounds and antioxidant activity with DPPH free radical scavenging and to measure the total phenol content of the hydro-alcoholic extract of aerial parts and root of *Campanula latifolia* collected from northern Iran (Figure 1).



Figure1. *Campanula latifolia*, Giant Bellflower

Methods

Plant material

Sample of *Campanula latifolia* was collected from southern heights of Sari city in Mazandaran Province on June 2015, and a voucher specimen (Code No. IRAN70100/1.) has been deposited in *Herbarium Ministrii Iranici Agriculturae* (Iran).

Extraction

After isolating the plant roots and shoots, the specimen was dried out in the open air and away from sunlight. 254 g of the powdered areal parts and 186 g of powdered root were extracted using 80% ethanol by soaking for 48 hours (three times). The extracts were concentrated using a rotary evaporator and dried using a vacuum oven.

Phytochemical assay

Qualitative phytochemical screening of primary and secondary metabolic compounds including saponins, flavonoids, alkaloids, steroids, and tannins were performed on the aerial parts and root extracts of *Campanula latifolia* according to the standard common phytochemical methods [11].

Total phenolic contents determination

Total Phenolic content of aerial parts and root extracts was determined using Folin-Ciocalteu reagent. 200 μ l from extracts was added to 1.5 mL of reagent that was 10 times diluted with distilled water and stored at 20 °c for 5 minutes. Then 1.5 mL of sodium bicarbonate solution (60 g/L) was added to the mixture. After 90 minutes at 22 °c, absorbance was measured at 765 nm by spectrophotometer; the tests were done three times. The phenolic content calibration curves was drawn by measuring the absorbance of some concentrations (25-150 mg/L) of Gallic acid as a standard and the results were indicated as milligrams of gallic acid equivalents (GAE) per gram of dry extract as means \pm SEM [12].

DPPH radical scavenging assay

The hydro alcoholic extracts of aerial parts

and root of *C. latifolia* were assessed for its free radical scavenging ability using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method according to Brand Williams et al. (1995). Three concentrations (1000, 2000, 3000 µg/ml) of sample solutions (1 mL) in methanol were added to DPPH methanol solution (2 ml, 40 µg/mL). After 30 min, the absorbance was measured at 517 nm. All experiments were performed in triple replicate. Percentage of radical scavenging activity of sample was calculated according to previous study [13].

Results

Ethno-botany of Campanula species

As shown in table 1, there are different types of campanula as vegetables in the menus of different countries such as Italy, Russia, and Turkey, as well as applications in the folk medicine of these areas. The root of the *Campanula rapunculus* is considered as a suitable diet for people with diabetes because it has more inulin than starch. In some parts of Italy, the roots of this plant are used in the mixed salads. The leaves of this plant are useful in decreasing the inflammation of the oral cavity, and also its herbal flower infusion is used for gargle (Table 1).

Table1. *Campanula* species and their folk medicine uses

Campanula species (used part)	Local name	Folk medicine uses	reference
<i>Campanula rapunculus</i> L. (flowers, leaves, roots or rootstocks, young shoots) <i>Campanula glomerata</i> L. (roots) <i>Campanula trachelium</i> L. (root)	Raponzolo, Rapacciola Ramponzolo, Raperonzolo, Rapunzoli, Mudar plant, Rampion	root sweet, leaves bitter; Taproots strips, leaves, are consumed in a mixed salad of wild herbs together with crushed hard- boiled eggs. Leaves used to treat warts, infusion of flowers used as a gargle to treat mouth and throat diseases and the oral cavity inflammation.	[14-18]
<i>Campanula wallichii</i> (plant)	Tootipat	Herb extract is used in wounds or cuts healing.	[19]
<i>Campanula trachelium</i>		They are consumed raw (in Turkey) as an appetizer, and for flavoring, in addi- tion to snacks at picnics and in travels.	[20]
<i>Campanula</i> spp		used for all inflammation of the mouth and throat.	[21]

The results of extraction

The concentrated aerial parts extract was very dark green, and the root extract was brown. The final extract of the aerial parts was 74.3 g, and the root extract was 27.9 g. The ratio of DER (Drug to Extract) with 80% ethanol solvent was calculated to be about 30% for the aerial parts extract and about 15% for the root extract.

Phytochemical assay

Preliminary phytochemical evaluation was performed on total extract of *C. latifolia*. According to results cardiac glycosides, sterols and triterpens are the main compounds of both aerial parts and root extracts (Table2).

Table2. Phytochemical assay of hydroalcoholic extract of aerial parts and root f *Campanula latifolia*

Sample	Anthocyanins	Tannin	Flavonoids	Saponins	Triterpens and sterols	Cardiac glycosides	Alkaloids
Aerial parts	-	-	-	-	+	+	-
Root	-	-	-	-	+	+	-

Total Phenol Content Evaluation

After obtaining a concentration of 1000 g / ml and its absorption readings for three consecutive days and three times in each of the three samples, it was concluded that the subsequent tested concentrations are 2000 µg / ml and 3000 µg / ml. Finally, using the standard gallic acid standard curve with the formula $Y = 0.006X + 0.022$, the concentration was calculated based on the average of the obtained data. Total phenolic content of hydroalcoholic extract from aerial parts and root were 0.053 and 0.037 (mg Gallic acid/g extract), respectively.

DPPH radical scavenging effect

Antioxidant effect of total extracts from *C. latifolia* aerial parts and root were evaluated with 2, 2-diphenyl-1-picryl-hydrazyl (DPPH). The re-

sults of the absorption of the samples and IC50 values (concentration of extract that can inhibit 50% of free radicals) are shown in Table 3.

According to results, IC50 was calculated 1419.354 for the root extract and 1163.636 µg / ml for the aerial parts extract.

Table 3. DPPH radical scavenging effect of hydroalcoholic extract from aerial parts and the root of *Campanula latifolia*

Extract concentration (µg/ml)	Absorbance (Mean±SD)	Percent of inhibition (%)	IC50 (µg/ml)
Aerial parts			
1000	0.358 ±0.037	36.971	1163.63
2000	0.057 ±0.006	89.612	
3000	0.048 ±0.015	91.549	
Root			
1000	0.345 ±0.007	39.260	1419.35
2000	0.181 ±0.008	68.133	
3000	0.173 ±0.015	69.542	

Discussion and conclusion

Campanula latifolia is used mainly as an ornamental plant for its beautiful appearance. In northern Iran, in the spring and before flowering, the leaves of the plant are eaten. By studying the basic phytochemicals and the compounds of the total extracts of root and aerial parts, and comparing it with other species of this genus, the primary compounds of this plant include the cardiac glycosides, triterpenes and sterols, and there are from small amounts of other chemical compounds in the plant.

Several phytochemical studies have been carried out on other species of *Campanula*. Dzhumyrko et al. (1984 to 1985) identified and isolated Cumarin and flavonoid compounds from some plants of this genus [22,23]. Yayli et al. (2005) reported triterpene compounds from *Campanula lactiflora* [24]. In a phytochemical study on *Campanula barbata*, phenylpropanoid compounds and a new catechin compound called barbatoflavan in addition to four flavon compounds have also been isolated. Barbatoflavan has shown a good DPPH radical

inhibitory effect [25]. In the phytochemical and antioxidant studies of *Campanula alliariifolia*, five phenolic compounds with quercetin-3-O-glucoside, quercetin-3-O-rutinoside, lobetyolin, kaempferol-3-O-glucoside, lobetyol were isolated and identified among which lobetyolin and lobetyol showed stronger antioxidant effects on DPPH radical inhibition than methanolic extract and other compounds [26].

In a paper published by Jaradat and Abualhasan in 2015, fifteen species of *Campanula*, collected from different parts of Palestine, were evaluated for the antioxidant effect through DPPH method. All studied species showed a significant antioxidant activity compared to trolox [27].

Qualitative assays have shown positive results suggesting the presence of cardiac glycosides in both of the studied extracts. As a result, the results of the experiments were evident at significantly lower concentrations. Positive results from the sterol and triterpene qualitative measurements can also guide more detailed phytochemical studies on the extract of this plant. However, quantitative studies have been carried out on

total phenol and antioxidants in the root, and aerial parts extracts of this plant and the results have shown low total phenol levels, and weak inhibition of free radicals, further studies are recommended on the therapeutic effects of the content of cardiac glycosides and steroids in this plant.

Conflict of Interest

None.

Acknowledgments

None.

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