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SHORT COMMUNICATION

Antioxidant potential assessment of phenolic and flavonoid rich fractions of *Clematis orientalis* and *Clematis ispahanica* (Ranunculaceae)

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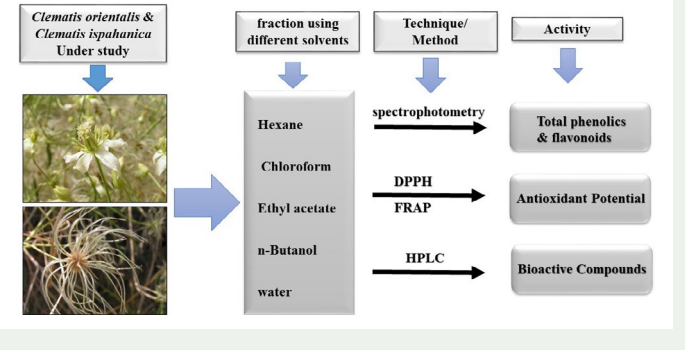
ABSTRACT

The antioxidant activities of crude extract fractions using Hexane, Chloroform, Ethyl Acetate, Butanol and Water of *Clematis orientalis* and *Clematis ispahanica* were investigated. 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and the ferric reducing/antioxidant potential (FRAP) were used to evaluate the antioxidant capacity. The total phenolics were found to be 4.37–9.38 and 1.32–11.37 mg gallic acid equivalents (GAE)/g in different fractions for *C. orientalis* and *C. ispahanica*, respectively. The ethyl acetate fraction of *C. orientalis* and chloroform fraction of *C. ispahanica* showed the highest DPPH and FRAP activities at a concentration of 300 µg/mL. The predominant phenolic compounds identified by HPLC in *C. orientalis* were Resorcinol (603.5 µg/g DW) in chloroform fraction and Ellagic acid (811.7 µg/g DW) in chloroform fraction of *C. ispahanica*.



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1. Introduction

The genus *Clematis* L. is the second largest genus of Ranunculaceae family comprises more than 300 species throughout the word (Wang 1999) and is distinguishable from other genera by the opposite leaves and usually viney habit (Tamura1995; Yang and Moor 1999; Wang & Bartholomew 2001). This perennial plant is abroad in the Northern hemisphere and different species are famous due to their usages in traditional medicines in the world. (Chawla et al. 2012). According to important botanical references (Boissier 1867; Rechinger et al. 1992; Wang and Xie 2007) different species of this genus have been introduced in Iran and some spices are known as 'Golak' or 'Kaf-kafak' in Persian. *Clematis orientalis* L. and *Clematis ispahanica* Boiss. are two major species of Iran which distributed in different localities of the country. Different plant parts of *Clematis* (roots and leaves) are traditionally used in various therapeutic uses as antibacterial, abirritative, analgesic, antiphlogistic, anticancer and diuretic agent. Also, crude extracts of *Clematis* showed diuretic, antimicrobial and anti-inflammatory activities (Li et al. 2009) and used in the treatment of rheumatic pain, fever, eye infections and many other human diseases. (Chawla et al. 2012) As we know, the bioactive effects of herbs are correlated with their phytochemical compounds. Thus, more qualitative and quantitative information about their chemical constituents will certainly help us to understand their pharmacological activities within the human body. Phytochemical investigation showed that a wide range of constituents were found in the different *Clematis* species. Triterpenoids saponins, flavonoids and lignans are tree major class of chemical constituents along with other alkaloids, steroids, coumarins, organic acids, macrocyclic compounds, volatile oils and polyphenol constituents (Hao et al. 2004; Chen et al. 2009; Du et al. 2010), while some triterpenoid saponins and alkaloids isolated from this genus showed cytotoxic, antibacterial and antifungal activities (Li et al. 2009). A noticeable cytotoxic effect of phytochemical compounds and extracts of various *Clematis* species were reported in distinct research projects (Rana et al. 2015; Chang et al. 2017). Reports on the chemical components of Iranian species of genus *Clematis* are scarce up to now and so it is the first report on the phytochemical content and their antioxidant properties in two *Clematis* species of Iran. Hence, the aim of this study was determined on the presence of various phenolic and flavonoid compounds by RP-HPLC as well as assessing the antioxidant activity of rich fractions of *C. orientalis* and *C. ispahanica*.

2. Results and discussion

2.1. Total phenolic and flavonoid compounds

The results of using different solvents for the extraction/fractionation of total phenolic and flavonoid content of *C. orientalis* and *C. ispahanica* are given in Table S2. From this Table, it was evident that plants extract contained noticeable amounts of extractable compounds. It is clear that the different solvents used for the extraction and fractionation of *C. orientalis* and *C. ispahanica*, had different abilities to extract substances from these medicinal plant. The estimation of phenolic and flavonoind content in *C. orientalis* among different fractions revealed that the ethyl acetate fraction exhibited higher phenol and flavonoid content of 9.83 mg/g GAE and 4.4 mg/g rutin followed by butanol fraction (6.74 and 2.66 mg/g) > chloroform fraction (5.8 and 3.2 mg/g) > hexane fraction (4.45 and 1.95 mg/g) > water fraction (4.34 and 1.85 mg/g), respectively. Whereas in *C. ispahanica,* chloroform fraction exhibited

higher phenol and flavonoid content of 11.37 mg/g GAE and 5.6 mg/g Rutin followed by hexane fraction (9.04 and 4.67 mg/g) > ethyl acetate fraction (4.78 and 1.53 mg/g) > butanol fraction (2.71 and 1.05 mg/g) > water fraction (1.32 and 0.87 mg/g), respectively. Total flavonoids and phenolic contents in chloroform and ethylacetate fractions were significantly higher than those of hexane, butanol and water fractions ($p < 0.05$). These results were similar to the previous report by Mariod et al. (2009) that phenolic content among different fractions of black cumin seedcake, surprisingly revealed that the ethyl acetate fraction exhibited higher phenol content of 78.8 ± 0.08 mg/g GAE followed by the water, methanol and hexane fraction, respectively. Ethyl acetate is often used as an extraction solvent with a significant selectivity in the extraction of low-molecular-weight phenolic compounds and high-molecular-weight polyphenols (Scholz & Rimpler 1989). These results are in good agreement with another study by Škerget et al. (2005), in which total polyphenolic content of olive leaf extract. Both flavonoid and phenolic compounds from olive leaf are known to have diverse biological activities and may also be responsible for the pharmacological actions of olive leaf or at least for synergistically reinforcing those actions (Abaza et al. 2007).

2.2. High-performance liquid chromatography analysis (HPLC)

The quantity of phenolic and flavonoid compounds of *C. orientalis* and *C. ispahanica* aerial parts extracted and its fractions are shown in Table S2 and S3. It showed that gallic acid, resorcinol and syringic acid were found to be a major bioactive compound in *C. orientalis*. However, the syringic acid and ellagic acid were the main bioactive compounds present in *C. ispahanica*. The overall results indicated that in *C. orientalis* the gallic acid as a main content in hexane and ethylacetate fractions were 367.5 and 373 µg/g DW, whereas those in chloroform and water fractions were 144 and 225 µg/g DW, respectively. In Butanol fraction of this species, gallic acid was not detected. On the other hand, in *C. ispahanica* the ellagic acid was most predominant compound were the higher amount in chloroform, ethylacetate, hexane and butanol fractions with respective values of 811.7, 670, 593.5 and 352.4 µg/g DW. This is the first time we are going to report the phenolic compounds of *C. orientalis* and *C. ispahanica*.

2.3. DPPH scavenging activity test

The DPPH scavenging activities of different extract/fractions of *C. orientalis* and *C. ispahanica* aerial parts were determined using method described by Gulcin et al. (2004). The results are shown in Table S4. The obtained results showed the scavenging effect of *C. orientalis* fractions on DPPH radical and was in the following order: ethylacetate (66.54%) > butanol (60.11%) > chloroform (58.42%) > hexane (55.52%) > water (52.36%) fractions. Meanwhile, the order of DPPH values for investigated *C. ispahanica* extract/fractions were chloroform (63.46%) > hexane (60.57%) > ethylacetate (57.04%) > butanol (53.11%) > water (48.53%). It was noted that among the different fractions, ethylacetate from *C. orientalis* and chloroform from *C. ispahanica* were found to be significantly higher DPPH radical scavenging activity at 300 mg/mL concentrations ($p < 0.01$) from other extract fraction, respectively. It was clear that the antioxidant potential of *C. orientalis* and *C. ispahanica* extracts and its fractions in DPPH assay was linearly correlated to their total phenolic compounds. The antioxidant activity increased proportionally to the polyphenol content and a positive linear relationship

between antioxidant activity and total phenolic and flavonoid compounds was found. Malencic et al. (2008) found that antioxidant activity of soybean seed extracts increased proportionally to the polyphenol content with a linear relationship between DPPH values and total polyphenols. In the same manner, Chew et al. (2008) found a correlation between the TPC and IC_{50} in edible seaweeds extracts and they mentioned that the high-level TPC gives low IC_{50} and results in high level of antioxidant capacity due to the high amount of polyphenolic constituents.

2.4. Reducing power activity assay

Table S5 shows the antioxidant activity by the FRAP method of different fractions of *C. orientalis* and *C. ispahanica* aerial parts when compared to the standard. It is apparent from the table that the ethyl acetate fraction from *C. orientalis* showed the highest antioxidant activity with a value of 64.13% followed by the butanol (58.5%), chloroform (56.62), hexane (54.21) and water fractions (50.36). However, the reductive potential of *C. ispahanica* extracts at a concentration of 300 µg/mL (Table S6) was found to be in the ascending order: chloroform > hexane > ethyl acetate > butanol > water, with respective values of 62.5, 59.13, 55.66, 50.11 and 45.36%. From the above findings, the results are well correlated with the amount of phenolic and flavonoid constituents which are present in the respective extracts.

3. Conclusion

It can conclude that all the fractions of the *C. orientalis* and *C. ispahanica* aerial parts showed different phenolic and flavonoid compounds as well as different antioxidant activities, reducing power ability, free radical scavenging activity since the different fractions of plant extracts exhibited different reROS scavenging activities, there may be different percentages of phytochemical constituents present in the fractions. Further studies needed to evaluate the *in vivo* potential of the fractions in different experimental animal models and the isolation and identification of the antioxidant principles are needed to evaluate by different methods.

Disclosure statement

No potential conflict of interest was reported by the authors.

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