Evaluation of phytochemical and antioxidant activity in different parts of *Heracleum gorganicum* Rech.F. in Golestan province of Iran

Masoumeh Mazandarani[1]*, Samira Makari [2], Gholam Reza Bajian[2], Parastoo Zarghami Moghaddam[3] and Mehdi Abrodi[4]

1. Department of Botany, Gorgan Branch, Islamic Azad University, Gorgan, Iran
2. Department of Botany, Shiraz Branch, Islamic Azad University, Shiraz, Iran
3. Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran
4. Young Researchers Club, Gorgan Branch, Islamic Azad University, Gorgan, Iran

Abstract

The aim of the present experiment was to study the relationship between secondary metabolites content (phenol and flavonoid) and antioxidant activity in root, stem, leaves, and fruits of *Heracleum gorganicum* Rech. F. The various parts of plant were collected in Ziarat Mountain in Golestan province. Total phenolics (TP) and total flavonoids (TF) contents were determined spectrophotometrically and antioxidant activity was measured by 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH) method. Results showed that the TP varied from 1.31±0.22 to 14.6±0.52 mg GAE g^{-1} in the parts and TF contents were between 2 ±0.5 to 84.84 ± 11.65 mg QUE g^{-1}. Antioxidant Activity (IC 50) was measured in ranges 0.11±0.01 to 0.23±0.015 mg ml^{-1} in DPPH method. There was a positive correlation between antioxidant activity and secondary metabolites content. The results of the present study showed that the leaves of *H. gorganicum* had the highest content of TP and TF compounds and antioxidant activity, providing natural sources for antioxidant compounds. This confirmed traditional uses of the plant by the rural healers as antiseptic, digestive, carminative and epilepsy in north of Iran.

Keywords: Heracleum gorganicum Rech. F.; antioxidant activity; flavonoids; phenols

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Introduction

Reactive oxygen species (ROS) such as superoxide, peroxides and hydroxyl radicals can induce changes in different biological tissues causing more than one hundred disorders in humans such as atherosclerosis, arthritis, ischemia, central nervous system injury, gastritis, cancer, AIDS, Alzheimer's, Parkinson's, and even in the aging process (Coruh et al., 2007; Dash et al., 2005). Therefore, antioxidant compounds are required for the protection against the oxidizing agents because these substances play main role in inhibiting and scavenging free radicals (Dash et al., 2005). Currently, many synthetic antioxidants used in the food industries show toxic properties

*Corresponding author
E-mail address: mazandarani.m@gorganiau.ir
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for human and animal health (Wangensteen et al., 2004; Tepe et al., 2006; Nickavar and Abolhasani, 2009). The ever-increasing application of medicinal and aromatic plants as natural source of antioxidant compounds in food and drug industries around the world is widespread (Kirca and Arslan, 2008). Therapeutic effects of several medicinal plants are related to their phytochemical components and their secondary metabolites such as phenolic compounds, flavonoids, alkaloids and tannins (Mohammedi and Atik, 2011), which have the ability to donate hydrogen atoms or electrons, chelate metal cations and ability to scavenge free radicals (Pietta et al., 1998). Polyphenols have been reported to have multiple biological effects including antioxidant activity (Nickavar and Abolhasani, 2009). Iran is a country with five major climates and high biodiversity that has been a unique land for growing more than 7500 plant species, many of them categorized as herbal medicine (Ahmadian-Attari et al., 2009). The genus *Heracleum* belongs to Apiaceae family with more than 125 species widely distributed in the world (Pimenov and Leonov, 2004; Tosun et al., 2008) and 10 species in the flora of Iran (Mojab and Nickavar, 2003). *Heracleum gorganicum* Rech.F. with locally known "Golpar" is used as flavouring agent and spice for food and making pickles in some areas of Iran. The fruits, young shoots, stem, seeds and leaves of this genus are used as carminative, antiseptic, digestive, epilepsy and analgesic in the Iranian folk medicine (Firuzi et al., 2010; Hajhashemi et al., 2009; Sayyah et al., 2005; Mojab and Nickavar, 2003). In Indian traditional medicine, decoction of the *Heracleum candolleanum* root is used against arthritis and paralysis (Chacko et al., 2000) also, various studies show that some *Heracleum* species are used as antipyretic, analgesic, diaphoretic (Taniguchi et al, 2005), for rheumatic disease, lumbago, gastralgia, and injuries from falls (Niu et al., 2004) hypertension (Eddouks et al., 2002), epilepsy (Eadie, 2004; Sayyah et al., 2005) and antiseptic (Souri et al., 2004; Sonboli et al., 2007). The roots of *Heracleum nepalense* are reported to have coumarins and steroids (Dash et al., 2005). Many kinds of secondary metabolites including furanocoumarins, anthraquinones, stilbenes, furanocoumarin dimers, flavonoids, hydrocarbons, oxygenated monoterpenes and sesquiterpene have been identified and isolated from various species of *Heracleum* (Kuljanabhagavad et al, 2010).

The main aim of the present study was to evaluate total phenolic and flavonoid contents, and antioxidant activities in different parts of *Heracleum gorganicum* Rech.F which has been used by the rural healers of Golestan province.

Materials and Methods

Plant Materials

Root, stem, leaves and fruit of *Heracleum gorganicum* were collected in north east of Golestan province at 2400 m altitude during March to July 2010. The voucher specimen was identified and deposited at the Herbarium Museum of Islamic Azad University, Gorgan branch. The plant organs were dried, and ground to a fine powder using a laboratory mill and the powdered materials were kept at room temperature (21–23 °C), and protected from light.

Extract preparation for phytochemical and antioxidant tests

In phytochemical tests, the dried roots, stem, leave and fruit of *Heracleum gorganicum* (5 g) were extracted overnight with 100 ml of methanol, in a mechanical shaker at room temperature. Each plant extract was filtered with Whatman No. 1 filter paper and stored at 4° C.

In an antioxidant test, 45 g of different parts (roots, stem, leave and fruit) of *Heracleum gorganicum* were extracted with 300 ml of methanol solvent in a mechanical shaker at room temperature. Extracts were filtered with Whatman No. 1 filter paper. The filtrates obtained from extract were then evaporated to dry at 40 ℃ in a rotary from evaporator and stored at 4 ℃ (Arabshahi-Deloue and Urooj, 2007).

Total phenols determination

Total phenolic content was estimated by the Folin Ciocalteu method, based on the procedure suggested by Pourmorad et al. (2006). Then 0.5 ml of plant extracts or Gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml) and aqueous Na_2CO_3 (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. Gallic acid was used as a standard for calibration curve. Total phenol values were expressed in terms of mg equal Gallic acid in 1 g dry plant powder.

Total flavonoids determination

Total flavonoids content were determined by aluminum chloride method (Pourmorad et al., 2006). Extract of different parts (0.5 ml) were separately mixed with 1.5 ml of solvent, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. They were kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. Quercetin was used as a standard for calibration curve. Total flavonoid values were expressed in terms of mg equal quercetin in 1 g dry plant powder.

Antioxidant activity assay

1,1-diphenyl-2-picryl hydrazyl radical scavenging capacity

The ability of the extract for free radical scavenging was assessed by the method suggested by Arabshahi-Delouee and Urooj (2007). Briefly, 1 ml of a 1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 12.5–1000 µg of dried extract). The mixture was then vortexes vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

Table 1
Comparison of secondary metabolites and antioxidant activity of different parts of *H. gorganicum*

Part / Test	Different parts of *H. gorganicum*			
	leave	Stem	Fruit	Root
Flavonoid (mg QUE g^{-1})	84.84±11.65	28.3±2.7	9.08±0.24	2±0.5
Phenol (mg GAE g^{-1})	14.6±0.52	3.94±0.66	3.62±0.55	1.31±0.22
DPPH IC50 (mg ml^{-1})	0.11±0.01	0.13±0.01	0.17±0.01	0.23±0.01

DPPH scavenging activity (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

Statistical analysis

For all assays, data were expressed as means ± S.E. and analysis was carried out using Microsoft Office Excel 2007. The student t-test was applied to test for significant differences and $P < 0.05$ was considered statistically significant.

Results

Total phenolics, flavonoids

The level of phenol and flavonoid compounds in different parts of *H. gorganicum* is shown in Table I. The finding indicated that the TP content of various parts of plant had significant variation, ranging from 1.31±0.22 to 14.6±0.52 mg $GAEg^{-1}$ and TF content 2 ±0.5 to 84.84± 11.65 mg $QUEg^{-1}$. The lowest content of these secondary metabolites was found in the root compared with the other parts, whereas the most contents were observed in leaves with TP (14.6 mg $GAEg^{-1}$) and TF (84.84 mg QUE g^{-1}). (Figs. I, II and Table I). Findings showed that leaves of plant with the highest content of TP and TF compounds could be used as an important part with high potency in scavenging of free radicals.

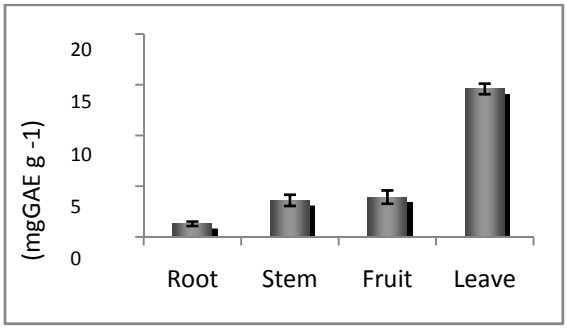

Fig. I. Total phenol contents of various parts *H. gorganicum*

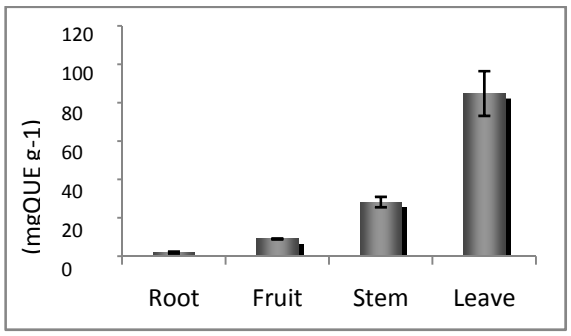

Fig. II. Total flavonoid contents of various parts *H. gorganicum*

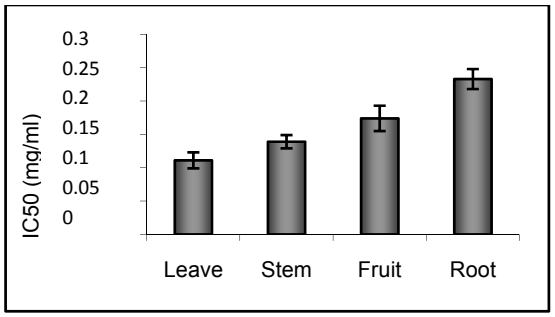

Fig. III. Amount of IC50 in various parts of *H. gorganicum*. in DPPH method

Through scavenging the radicals, antioxidants are useful for the treatment of more than one hundred disorders in human body such as arthritis, atherosclerosis, ischemia, gastritis, cancer, neurodegenerative and AIDS (Pourmorad et al., 2006).

Figure III and table I show antioxidant activity by inhibition of the free radicals. IC 50 contents in various parts of *H. gorganicum* in DPPH method was 0.11±0.01 to 0.23±0.015 mg ml^{-1}. Among the organs under study, leaf extract with the lowest contents of IC_{50} (0.11 mg ml^{-1}) had the most potent antioxidant by inhibition of the free radicals when compared with the other parts of the plant, whereas roots had the highest content of IC_{50} and the weakest antioxidant activity (Fig. III and Table I).

Discussion

Free radicals are involved in diseases like cancer, AIDS and neurodegenerative (Pourmorad et al., 2006) and antioxidant compounds through their scavenging power are useful for management of different diseases.

Previous studies indicated that the antioxidant activity of phenolic and flavonoid compounds is attributed to their redox properties, ability to chelate metals and quenching of singlet oxygen (Kessler et al., 2003, Surveswaran et al., 2007).

In their study, Dash et al., (2005) reported the DPPH scavenging capacity of the *H. nepalense* extract on the order of 72.38 ± 3.92 % at 1000 µg ml^{-1}. Results of Nickavar et al., (2009) on *H. persicum* showed that the total flavonoid content and IC50 values were 22.23 µg mg^{-1} and 294 µg ml^{-1} respectively. The DPPH scavenging (IC50) and total phenol content of methanol extract for *H. persicum* Desf. Were reported 0.438 mg ml^{-1} and 59.6 GAEs µg mg^{-1} respectively (Coruh et al., 2007). In another study, total phenolic content of *H. persicum* indicated 1.35 mg catechin equivalent g^{-1} essential oil and IC50 was 7.4 mg ml^{-1} (Firuzi et al., 2010).

Our study showed that the high contents of phytochemical compounds (phenol and flavonoid) in *H. gorganicum* can explain its high radical scavenging activity.

Findings indicated that there was direct correlation between total phenol and flavonoid contents (14.6 mg GAE g^{-1} and 84.84 mg QUE g^{-1} respectively) and antioxidant activity (0.11 mg ml^{-1}) for leaves extract of *H. gorganicum.* These results were consistent with the findings of many research groups who reported such direct relationships between total phenolic content and antioxidant activity (Cai et al., 2004; Pourmorad et al., 2006; Tawaha et al., 2007; Kirca and Arslan, 2008).

Also in other studies about variety of species (*Sylibum Marianum*, *Lithospermum erythrorhizon* , *Cordia multispicata* , *C. myxa* and *Tournefortia bicolor, Ehretia laevis,* and *Borago officinalis*),

were observed similar results (Mokarram shah et al., 2011; Hadaruga, 2009; Cai et al., 2004; Conforti et al., 2008).

The results of the present study showed that the leave of *H. gorganicum* with the highest content of TP and TF compounds and antioxidant activity as an important part of this plant. Therefore, high potency in scavenging of free radical may be related to the high amount of secondary metabolites in this organ, which could provide natural sources of antioxidant compounds to treatment of disorders associated with free radicals that these study may be of value for future research and also confirmed to traditional uses by the rural healers as antiseptic, digestive, carminative and epilepsy in north of Iran.

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