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ARTICLE

Antioxidant Activity and Flavonoid Content of *Matricaria chamomilla* Extracts from Different Populations of Iran

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ABSTRACT

Matricaria chamomilla is a valuable medicinal plant belonging to the *Asteraceae* family. Its medicinal and pharmaceutical impacts are correlated to major flavonoid compounds like apigenin. In this investigation, methanolic extracts of *M. chamomilla* inflorescence gathered from six natural populations were evaluated for their phytochemical content and antioxidant activity. The content of total flavonoid and phenol modified from 3.72 to 7.94 mg g-1 DW and 1.37 to 3.51 mg g-1 DW, respectively. Flavonoid compositions revealed significant differences among six populations, and the highest apigenin (1.27 % (w/w)) and apigenin-7-glucoside (0.86 %(w/w)) contents were recognized in MD populations, respectively. Both PCA and Pearson's correlation analyses revealed total phenol, flavonoid, apigenin and apigenin-7-glucoside were negatively correlated with the IC50 of DPPH activity and EC50 of reducing power. Altitude and precipitation indicated the positive and negative effects on phytochemical contents, respectively. These results can provide a theoretical basis for getting the targeted antioxidant phytochemicals of *M. chamomilla* for pharmaceutical and food industries, and also give a science for selection of the best population for cell culture and secondary metabolite production in future.

1. Introduction

Reactive oxygen species (ROS) could cause oxidative injuries of protein, DNA, and cell membrane which are associated with several chronic diseases such as inflammation, tumor, cardiovascular disease and so on [1]. Plants produce several bioactive compounds which act in plant defense mechanisms to neutralize ROS in order to keep away from oxidative damage. Natural antioxidants can also preserve the human body from free radicals and the progression of certain chronic diseases [2] and is often supposed to be safe for eating, due to their plant source [3], but this may alter based on the plant species and environmental factors that influence growth. Natural antioxidants exist in divers' parts of medicinal plants have been indicated to have potential applicants for antioxidant, antiarthrogenic, antiallergenic, antiinflamatory, antithrombotic, antimicrobial activities [4,5]. Also, researchers are scanning for natural antioxidants as an alternative to unnatural antioxidants, such as butylated hidroxyanisole, *ter*-butylhidroxyquinone, and butylated hydroxytoluene which

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are extensively used in the food and pharmaceutical industries. Attention to plant-derived food additives has risen, because the usage of unnatural antioxidants has been related to the potential health risks, so it produced severe regulations above their use in foods *Matricaria chamomilla* is a medicinal plants belonging to Lamiacea family. It is an herbaceous annual plant growing up spontaneously to 5-80 cm, inhabits in various areas of Iran with white ray flowers. The presence of flavonoids apigenin has been previously indicated in *M. chamomilla* [6]. Apigenin has extensive biological activities including anti-inflammatory, antioxidant, anti-tumor, neuro-protective, anti-allergic and anti-microbial activities [7,8,9,10,11]. The phytochemical composition of Iranian *M. chamomilla* has been examined in some researches, while less consideration has been acted to compare different populations for application in the plant tissue culture, production of valuable secondary metabolites as well as in food and pharmaceutical industries. A few studies have described about total flavonoid, phenol content, and antioxidant activity in *M. chamomilla* aerial parts from Kashan vicinity [12]. Many studies reported the notable variations in phytochemical composition and antioxidant activity in different geographical places. For instance, notable differences in the total flavonoid, total phenolic contents, and antioxidant activity were indicated in C*yclocarya paliurus, Alternanthera sessilis, Basella alba, Rhus* and *Ginkgo* extracts which collected from various geographical areas [13,14,15,16]. Difference in phenolic compounds and antioxidant capacity were also reported in pyrola gathered from different areas [17]. There is a few data about phytochemical compounds of different population of Iranian *M. chamomilla* [12], and hasn't compared among until now. To our best knowledge, no documented data report on different contents of phenolic and flavonoid, and antioxidant capacity in various populations of chamomile in western and center provinces of Iran. The aim of the present study was to investigate the bioactive compositions and antioxidant capacity of *M. chamomilla* extracts, gathered in six areas of Iran at flowering stages.

2. Materials and Methods

2.1 Plant Material and Site Description

A total of 200 samples of *Maricaria chamomilla* were gathered from six natural populations (the cities of Bonab, Andimeshk, Tehran, Kermanshah, Esfahan and Yasuj) in August 2017 (Table 1). All the plants originated in the areas had a similar phenotype. The data of location were measured by a Global Positioning System (GPS, Vista Garmin) receiver (Table 1).

Table 1. Geographical site, altitude and precipitation of different populations of *M. chamomilla*

Population	Collection site	Altitude (m)	Precipitation (mm)
MA	Andimeshk, Khozestan Province, Iran	700	438
MB	Bonab, East Azarbayjan Province, Iran	1313	300
ME	Esfahan, Esfahan Province, Iran	1585	145
MD	Dizin, Tehran Province, Iran	2500	241
MK	Kermanshah, Kermanshah province, Iran	1319	441
MY	Yasuj, Kohgiluyeh and Boyer-Ahmad Province, Iran	1855	850

2.2 Preparation of the Methanolic Extracts

M. chamomilla inflorescence was separated from the stem and dried. The sample was powdered into a pot and 0.5 g exactly was placed into an Erlenmeyer flask (100 ml). 50 ml of methanol (80%) was added and the sample was left to soak overnight. Then, the mixture was sonicated in 37 KHz and 35 ºC, and filtered by a Whatman filter paper. The filtered solution was volatilized under decreased pressure (Rotavapor, T <38 °C) and the extract was dried in a room temperature by a vacuum, to steady weight. Extract was weighted and dissolved in methanol (500 µl), and the solution was placed at -20°C [18].

2.3 Total Phenolic Content

Total phenolic content was quantified by a Folin-Ciocalteu procedure method [19]. Methanolic extract (0.1 ml) was added to Folin-Ciocalteu reagent (2.5 ml). The reaction mixture was neutralized by sodium bicarbonate (7%). The mixtures were permitted to stay for 1 h, and the absorbance was registered at 765 nm. A calibration curve was provided by a standard gallic acid (GAE) solution, and indicated as mg GAE g^{-1} extract.

2.4 Total Flavonoid Content

Flavonoid content was quantified by a described method of Hatamnia et al. [20]. Briefly, the extract (0.5 ml) were added to the methanol (1.5 ml), 10% aluminum chloride (0.1 ml), 1M potassium acetate ((0.1 ml), and 2.8 ml distilled water, and the mixture was put at room temperature for 30 min. The absorbance was immediately read at 518 nm. A calibration curve was provided by a standard rutin (RU) solution, and indicated as mg RU/ g extract.

2.5 Determination of Apigenin by HPLC

The inflorescence of *M. chamomilla* (0.5 g) was extracted by methanol aqueous solution (20 ml), with bath ultrason-

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ic and overnight incubation at room temperature. After centrifugation at 5,000 rpm, the supernatant was collected, dried and resolved in methanol (500µL). For the qualitative and quantitative calculation of apigenin and derivate, HPLC program provided with a UV-Vis photodiode-array detector (Agilent Technologies 1260 infinity, Santa Clara, CA). The chromatographic separation was acquired by a C18 column (MZ Analysentechnik, Mainz, Germany). The motile phase (A solvent) was deionized water with 0.1% (*v/v*) phosphoric acid and non-motile phase (B solvent) was acetonitrile. The gradient system was 18% B (0-30 min), 67% B (30-60 min) and 18% B (60-65 min). The flow rate was 1mL/min at 25°C, and injection volume was 20 µL. The apigenin were identified by the absorbance peaks at 330 nm [21].

2.6 DPPH Radical Scavenging and Reducing Power Assay

The antioxidant capacity was examined by the estimation of the free radical-scavenging impact on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals [22]. Various concentrations of metanolic extract were added to 2.5 mL of DPPH (0.5 mM in methanol). The absorbance of the solution was read at 517 nm after 30 min, and the scavenging activity was measured as described methods of Patro et al. [23].

Colorimetric method was used to determine reducing power. Different concentrations of methanolic extracts were added to 2.5 mL potassium ferricyanide (10%) and 2.5 mL phosphate buffer (0.2 M, PH 6.6). The reaction mixture was placed for 20 min at 50 °C, and 2.5 mL trichloroacetic acid was added to the reaction solution. Then, the 2.5 mL solution was mixed with 2.5 mL distilled water and 0.5 mL ferric chloride (1%). The absorbance was read at 700 nm, and reducing power was calculated based on the method of Xia et al. [24].

2.7 Statistical Analysis

Statistical data analyses were performed using one-way ANOVA in triplicate replications, and mean comparison was measured by Duncan's multiple range tests at a significance level of P≤0.05 and values were indicated as means ± SD (standard deviations) using SPSS (Version 21). Principal component analysis (PCA) was used for estimating correlation between each variable by online CIMminner and XLSTAT (2016) software, respectively.

3. Results and Discussion

The present study was managed to examine the phytochemical contents in six populations of *M. chamomilla* and determine the effects of geographical and climate factors on antioxidant activity.

3.1 Total Phenolic and Flavonoid Contents

Total phenolic of the extracts from the inflorescence parts of six *M. chamomilla* populations are presented in Table 2. The results showed various amounts of total phenolic content from 7.94 to 3.72 (mg GAE g^{-1} DW) among different populations. The population of MD with 7.94 mg GAE g^{-1} DW showed the highest content of total phenolic. Total flavonoid content showed a significant difference ($p \leq 0.05$) among populations. The population of MD with 3.51 mg RU g^{-1} DW showed the higher content of total flavonoid than the other populations. Phenolic and flavonoid compounds are the intermediates of phenylpropanoid pathway which have several roles in plants, including antioxidant activity through transfer of single electron and hydrogen atom which involves in defense mechanisms against abiotic stresses [25,26]. Variation in total phenolic and flavonoid contents could be related to genetic variation of various populations which induced by environmental and genetic factors [27,28].

Table 2. Total phenol and flavonoid contents and antioxidant activity in different populations of *M. chamomilla*

Populations	Total phenolic (mg GAE g^{-1} DW)	Total flavonoid (mg RU g^{-1} DW)	IC50 of DPPH free radical (µg ml^{-1})	EC_{50} of reducing power (µg ml^{-1})
MA	5.27 ± 0.25^{d}	2.21 ± 0.052^{b}	29.56 ± 0.142^{b}	0.63 ± 0.023^{b}
MB	5.99 ± 0.32^{bc}	2.98 ± 0.076^{ab}	21.75 ± 0.122^{c}	0.25 ± 0.032^{c}
ME	6.88 ± 0.27^{b}	2.46 ± 0.093^{b}	20.65 ± 0.092^{c}	0.58 ± 0.012^{b}
MD	7.94 ± 0.43^{a}	3.51 ± 0.12^{a}	19.23 ± 0.081^{d}	0.21 ± 0.041^{c}
MK	3.72 ± 0.37^{cd}	1.37 ± 0.083^{c}	73.35 ± 0.158^{a}	1.04 ± 0.033^{a}
MY	5.15 ± 0.19^{c}	2.56 ± 0.067^{b}	35.89 ± 0.165^{b}	0.72 ± 0.029^{b}

Values are given as mean ± SE (n = 3) in each group. Different letters indicate significant differences at $P \leq 0.05$ (LSD).

3.2 Apigenin and Apigenin-7-glucoside Content

Significant differences were observed among six populations for flavonoid compositions. The content of apigenin and apigenin-7-glucoside were identified ranged from 0.08 to 1.27 (% W/W) and 0.009 to 0.84 (% W/W) in different populations, respectively. The population of MD showed the maximum apigenin (1.27%) and apigenin-7-glucoside (0.86%) contents, respectively. Moreover, MK population showed the trace amount of apigenin and apigenin-7-glucoside. Flavonoids scavenge free radicals due to their special chemical structures, and react with

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different reactive oxygen species and metal chelating [2,3,5]. The previous study showed the content of 0.74% (w/w) apigenin in wild *M. chamomilla* aerial part [12]. Flavonoid biosynthesis is controlled by divergent expression of genes regarding to the stages of plant development and ecological aspects [24,25]. In some study reported that Nei's gene diversity (h) and Shannon's information index (I) of *M. chamomila* was the highest in Khuzestan province (h = 0.364 and I= 0.528) and lowest in Fars province (h = 0.16 and I= 0.23) of Iran [28]. Pearson's correlation coefficients analysis displayed the positive effect of altitude and negative effect of perception on apigenin and apigenin-7-glucoside contents (Table 3). It showed that high altitude and low annual perception could induce the accumulation of the especial flavonoid compounds.

Table 3. Pearson's correlation coefficients between the phytochemicals and antioxidant activity in *M. chamomilla* aerial part

Index	Total phenolic	Total flavonoid	Apigenin	Apigenin-7-glucoside
IC_{50}	-0.852**	-0.831**	-0.911**	-0.652**
EC_{50}	-0.846**	-0.897**	-0.895**	-0.858**
Altitude	0.615**	0.603**	0.486*	0.279
Precipitation	-0.578*	-0.263	-0.422	-0.393

Note: * and ** indicate significant effects at $p \leq 0.05$ and $p < 0.01$, respectively.

3.3 Antioxidant Activity

For determination of antioxidant activity, the analyses of DPPH radical scavenging capacity and reducing power were performed in the *M. chamomilla* inflorescence, and a significant difference were indicated in antioxidant activities among various populations. The IC50 amounts of DPPH scavenging activity was ranged from 19.23 to 73.35 µg ml^{-1} in different variation (Table 2). MD population showed the lowest IC_{50} capacity with high antioxidant capacity, while MK population showed poor antioxidant level. Moreover, the EC_{50} amounts of reducing power were different from 0.21 to 1.04 µg mL^{-1} among in population and the highest capacity of reducing power was shown in MB and MD populations. In some study reorted that the IC_{50} level of *M. chamomilla* in ethanolic extract was 26.7 µg ml^{-1} [29],which showed lower antioxidant capacity than the extract indicated in this study, and probably related to the extraction method and geographical factors. Developmental stages, environmental factors, and genotype could affect the accumulation of secondary metabolites and the antioxidant capacity [17].

3.4 Relationships between Phytochemicals and Antioxidant Activity

Pearson's correlation analysis was performed to clarify the probable relation between phytochemical accumulation and antioxidant activity in *M. chamomilla* inflorescence (Figure 1). The PCA test identified 82.74% of the total variation with 69.16.0% in axis 1 and 13.58% in axis 2 (Figure 2). Based on the PCA analysis, the IC50 of the DPPH, EC50 of reducing power and precipitation were negatively related to total phenol, flavonoid, apigenin and apigenin-7-glucoside variables. Indeed, all the phytochemical compounds were positively related to antioxidant capacity. It found that IC50 and EC50 variables act a vital role in the second axis of PCA test.

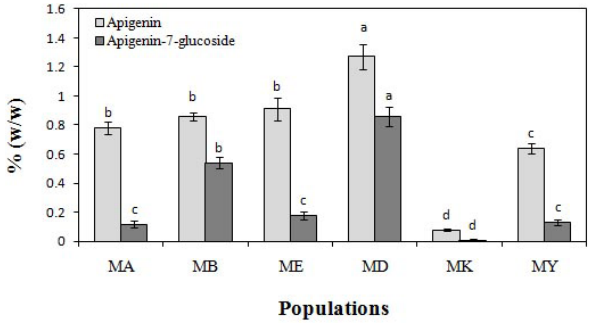

Figure 1. The content of apigenin and apigenin-7-glucoside of *M. chamomilla* in different populations

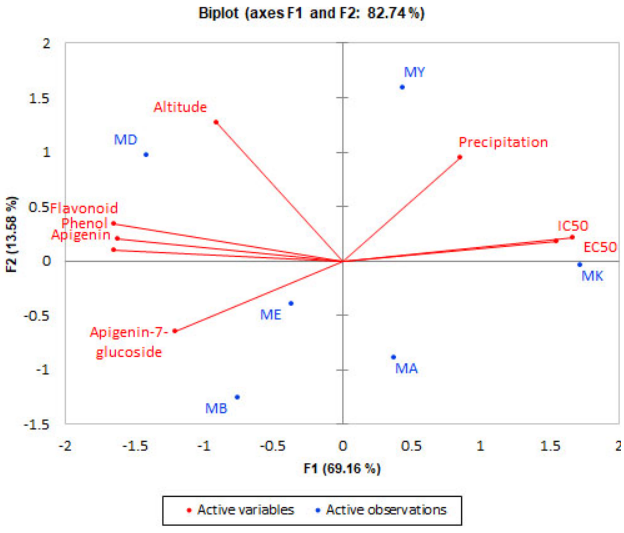

Figure 2. PCA of bioactive compounds and antioxidant activity in *M. chamomilla*

4. Conclusion

Significant differences in antioxidant activity and phytochemical compositions were identicated in metanolic extracts of *M. chamomilla* gathered from six natural pop-

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ulations. The contents of total polyphenol, total flavonoid, apigenin and apigenin-7-glucoside were significantly related to antioxidant ability, and the MD population showed the highest antioxidant activity. Our results introduce MD population for tissue and cell suspension culture and use its extract for pharmaceutical purposes.

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