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EFFICIENCY OF GREEN EXTRACTION BY AQUEOUS GLYCEROL ON ANTIOXIDANT AND ANTIRADICAL PERFORMANCE OF DANDELION (TARAXACUM OFFICINALE) AERIAL PART

Kevser Karaman^{1⊠}, Mahmut Kaplan², Rabia Say³, Serap Köprü³, M. Mücahit Yılmaz^{3,4}

¹Erciyes University, Faculty of Agriculture, Department of Agricultural Biotechnology, Kayseri, Turkey

²Erciyes University, Faculty of Agriculture, Department of Field Crops, Kayseri, Turkey

³Dr.Yılmaz Medicinal Plants and Drug Raw Materials Company, Kayseri, Turkey

⁴Dr. Yılmaz Clinic, Kayseri, Turkey

[™]kevserkaraman@erciyes.edu.tr

Article history:	ABSTRACT				
Received,	In this study, aerial parts of the dandelion were exposed to extraction by				
22 December 2020	different solvents such as water, ethanol, methanol and glycerol and also				
Accepted,	their aqueous mixtures to compare the effect of extraction solvents on				
25 December 2021	bioactive performance of the dandelion and also to show the effectiveness				
Keywords:	of hydroglycerolic extraction which is a green extraction process. Total				
<i>Hvdroglvcerolic extraction;</i>	phenolic content (TPC) and total flavonoid content (TFC) of the extracts				
Antioxidant:	were determined and also antiradical scavenging activities and antioxidan				
Antiradical:	capacities of the samples were also evaluated. TPC and TFC of the samples				
Bioactivity:	ranged between 4.63-21.28 mg GAE/g and 1.16-14.38 mg CE/g,				
Dandelion.	respectively. The highest TPC and TFC values were determined in aqueous				
	extract of glycerol (75% w/w) compared to other solvents. Additionally,				
	ABTS ⁺ and DPPH radical scavenging activity and ferric reducing capacity				
	and antioxidant capacity values were determined for the extracts and the best				
	solvent was also aqueous glycerol (75% w/w).				

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1.Introduction

Taraxacum officinale is one of the most popular medicinal plants belonging to the family of Asteraceae and known as dandelion which is a perennial plant and it was reported that the dandelion was rich in some flavonoids, triterpenes, coumarins, and phytosterols (You et al., 2010). It has been used in folk medicine for many years to threat fever, lactating and sore (Sun *et al.*, 2014). In manv throat pharmacological researches, it was showed that dandelion extracts the showed strong antioxidant, anti-inflammatory, anti-fertility and antitumor activities (Jeon et al., 2008; Park et al., 2010). In different studies, different parts of the dandelion have been investigated and their antioxidant properties (Hu and Kitts, 2005, Park et al., 2011), antimicrobial activities (Ionescu et al., 2013; Rodino et al, 2015; Oseni and Yussif,

2012) and antidiabetic properties (Hussain *et al.*, 2004) were reported.

In many studies, solvent extraction was performed using some effective organic solvents such as ethanol, methanol, acetone, ethyl acetate, n-hexane etc. to evaluate the bioactivity of the medicinal plants (Ghaima et al., 2013; Oseni and Yussif, 2012). As is known, extraction of the bioactive substances from the plant structure is the main and important process for the medicinal and aromatic plants and the quality and quantity of the bioactive compounds depend on the selected extraction solvent and extraction process (Lucchesi et al. 2004). In recent years, the use of green solvents such as glycerol which is environment friendly matter increased because they were evaluated as good alternative to the synthetic and organic

chemicals, and they showed better yield and quality of the extracts (Azmir et al. 2013). Glycerol is a natural, non-toxic, biodegradable and recyclable viscous liquid which is produced from renewable sources (Wolfson et al., 2006). It shows no easy flammability due to very high boiling point (290 °C) and it is really cheap solvent compare to other organic ones (Paleologou et al. 2016). Apostolakis et al. (2014) reported that the glycerol can favorably alter the polarity of the water and so, it can act as an effective co-solvent to increase the polyphenolic substance from the plant structure. In the literature, some studies regarding the efficiency of water/glycerol extracts were performed (Apostolakis et al. 2014; Karakashov et al. 2015a, Karakashov et al. 2015b; Eyiz et al., 2020). Taking into account all of this information, this study was planned to show the efficiency of hydroglycerolic extraction of dandelion aerial parts on some bioactive parameters. For this purpose, effect of hydroglycerolic extraction different at concentrations (25, 50 and 75% w/w) was compared hydroethanolic to and hydromethanolic extractions in terms of antiradical and antioxidant activities for dandelion.

2. Materials and methods

2.1. Materials

The dried aerial parts of dandelion (*Taraxacum officinale*) were procured from Karakaş Food Plant Co. (İstanbul). The moisture content of the dandelion was 9.93%. Glycerol was purchased from a local supplier in Turkey and ethanol and methanol were provided from Merck (Germany).

2.2. Plant extraction process

Two g of ground plant sample was weighed. Then 60 mL of solvent (glycerol, ethanol and methanol) at different concentrations (25, 50 and 75 and 100% w/w) as shown in Table 1 was incorporated into the sample and all samples were placed in shaking water bath to extract at room temperature (25 ± 0.5 °C) for 1 hour. After the process, the samples were centrifuged at 9000 g and 10 °C for 5 min and the supernatant was filtrated using 0.45 μ m and then the extract samples were stored for further analysis.

2.3. Analysis of total phenolic content (TPC)

TPC of the samples was determined using the method suggested by Singleton and Rossi (1965). For this purpose, 200 µl of the extract was mixed with 1800 µl of distilled water. Then 1 mL of diluted (1/10) Folin Cioceltaue reagent and after 1 min later, 2 mL of sodium carbonate (2% w/v) was added into all tubes. The samples were incubated for 2 hours at room temperature and dark conditions. At the end of the incubation, the absorbance values of the samples were recorded at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Total phenolic content of the samples was calculated as mg GAE/g sample using a calibration curve.

2.4. Analysis of total flavonoid content (TFC)

TFC of the samples was measured according to method of Zhishen *et al.* (1999). For this aim, 0.5 ml of the sample was mixed with 2 mL of distilled water and then 150 μ l of sodium nitrite (5% w/v) was added into the tubes and the samples were waited for 5 min. Then 150 μ l of AlCl₃ (5% w/v) was incorporated into the samples and after 6 min waiting, 1 ml of NaOH (1 M) and 1.2 ml of distilled water was added and the final mixture was vortexed and the absorbance of these mixtures was recorded at 510 nm by a UV-Vis spectrophotometer (Shimadzu, Japan) and the total flavonoid content of the samples was calculated as mg catechin equivalent (CE)/g sample.

2.5. Determination of DPPH radical scavenging activity

DPPH radical scavenging activity of the samples was determined as described by He *et al.* (2016). A 100 μ L of the extract sample was mixed 3900 μ L of DPPH radical solution in methanol (2 mM) and mixed well using vortex. After the incubation of the samples at room conditions in a dark place for 30 min, the absorbance values were recorded at 517 nm by a UV-Vis spectrophotometer (Shimadzu, Japan). DPPH radical scavenging capacity was calculated as % inhibition using the following Equation 1): % Inh. (Remaining) = 100-[(Abs_{control}-Abs_{sample}) /Abs_{control}]x100 (1)

2.6. Determination of ABTS⁺ radical scavenging activity

Firstly, ABTS⁺ radical was produced by preparation of ABTS⁺ stock solution. For this purpose, 7 mmol/L ABTS.+ stock solution was prepared and mixed with 2.45 mmol/L potassium persulfate. It was kept at dark conditions at room temperature for 16 h to complete the radical occurrence. At the end of the time, the stock radical solution was diluted with the buffer solution (pH 7.4) until the absorbance value of 0.7±0.05 at 734 nm was obtained. four different After that. concentrations (15, 30, 45 and 60 µL) of diluted extracts (1:20) were placed into the spectrophotometer cuvettes and 2 mL of ABTS⁺ solution was placed into the cuvettes having extracts and the samples were incubated for 6 min and the absorbance values of the samples recorded at 734 nm using were a spectrophotometer (Shimadzu, Japan). The radical scavenging activity of the samples as % inhibition was calculated using the following equation (Eq.2).

% Inh. = 100- [(Abs_{control}-Abs_{sample})/Abs_{control}]x100 (2)

where Abs_{sample} is the absorbance of $ABTS^{+}$ with sample; $Abs_{control}$ is the absorbance of $ABTS^{+}$ without sample. The % inhibition values were converted into the Trolox values and all results were expressed as Trolox equivalent antiradical capacity (µg TEAC/g sample) (Gong *et al.*, 2012).

2.7. Ferrous ion chelating activity

Iron chelating activities of the samples were determined according to the method suggested by Rival *et al.* (2001). For this purpose, 1 mL of the sample extract diluted as 1/10 was taken and 3.7 mL of ethanol (95% v / v) was added. Then, 100 µL of FeCl₂ was added to the samples and

immediately after vortexing the samples, $200 \ \mu l$ of ferrozine (5 mM) was incorporated into the tubes. The homogeneously mixed samples were allowed to incubate for 10 min at room temperature in the dark and the absorbance values of the samples were measured by UV-Vis spectrophotometer (Shimadzu, Japan) at 562 nm. Iron chelating activity values of the samples were calculated as % inhibition using the following equation.

% Chelating activity= 100-[(Abs_{control}-Abs_{sample})/Abs_{control}]x100 (3)

2.8. Ferric reducing antioxidant activity

Reducing power, which gives an idea about the antioxidant capacity of the samples, was determined based on the method applied by Malomo et al. (2011). One ml of the sample extracts of various concentrations and standard (ascorbic acid) were mixed with 2.5 ml of 0.2 M phosphate buffer solution (pH = 6.6) and then 2.5 ml of 1% w/v potassiumferricyanide [K₃Fe (CN)₆] was added. The samples were incubated for 20 min at 50 °C. After this step, 2.5 ml of trichloroacetic acid (10% w/v) was added to the reaction mixture and centrifuged at 1000 g for 10 minutes and 2.5 ml was taken from the top of the solution. 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl₃ were added to the separated part of the solution (2.5 mL) and the samples were mixed by vortex. Then the absorbance values of the samples were measured by UV-Vis spectrophotometer (Shimadzu, Japan). The results were given in mg ascorbic acid equivalent (mg AAE / kg).

2.9. Antioxidant capacity by phosphomolybdenum reduction

Antioxidant capacity of the samples was also evaluated by phosphomolybdenum reduction assays according to Prieto *et al.* (1999). In this regard, 400 μ L of extract was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Then the tubes were mixed and placed in a water bath to incubate at 95 °C for 90 min. At the end of the incubation, the tubes were cooled in an ice bath and the absorbance of the samples was recorded at 695 nm using UV-Vis spectrophotometer (Shimadzu, Japan). The results were expressed as mg ascorbic acid equivalent (mg AAE/kg) using a calibration curve created by ascorbic acid standard.

2.10. Statistical analysis

Statistical analysis of the data was evaluated using Windows based SAS 8.2 statistical analysis software (SAS Institute, Cary, North Carolina, USA). Duncan multiple comparison was performed with the significance level of 95%. All the analyses were carried out in duplicate with four repetitions.

Table 1. Solvent type and mixture ratios (g/g) used for the extraction

Solvent type	W	GLY	EtOH	MeOH
GLY	-	100	-	-
GLY-75	25	75	-	-
GLY-50	50	50	-	-
GLY-25	75	25	-	-
EtOH	-	-	100	-
EtOH-75	25	-	75	-
EtOH-50	50	-	50	-
EtOH-25	75	-	25	-
MeOH	-	-	-	100
MeOH-75	25	-	-	75
MeOH-50	50	-	-	50
MeOH-25	75	-	-	25

GLY: Glycerol, EtOH: Ethanol, MeOH: Methanol

3.Results and discussions

3.1 Total phenolic and flavonoid content of dandelion extracts

TPC of the samples was illustrated in Fig.1 and it was seen that the TPC of the samples was in the range of 4.63-21.28 mg GAE/g sample. Solvent type affected the TPC of the samples significantly (p<0.05) and the highest TPC was recorded for the extract obtained by 75% glycerol (Glycerol: Water 75:25 w/w) while the lowest TPC was for sole glycerol. Ethanol and methanol and also their aqueous mixtures

showed lower TPC compared to mixture of glycerol and water. Sole water or ethanol also showed similar TPC for the samples. As is seen from the Fig.1, there were no huge differences between 25, 50 and 75% for ethanol and methanol. This situation was also reported by Amyrgialaki et al. (2014) as both 40 and 60% levels of ethanol showed statistically similar TPC values for the samples. Rodino et al. (2015) reported the TPC of dandelion aerial parts was in the range of 15-19 mg GAE/g sample according to the extraction process type. Eyiz et al. (2020) investigated the bioactivity of red grape pomace extracted by aqueous glycerol and they reported that the TPC of the samples increased significantly with the increase of glycerol concentration (p<0.05). It was reported that the TPC of red grape pomace was measured as 16 mg GAE/kg for 10% glycerol level while it was 25.4 mg GAE/kg for the sample extracted by 50% glycerol. Increase of glycerol by fivefold provided an increase in TPC by 50% approximately. In another study, eggplant peel was extracted using an aqueous glycerol by ultrasound application and it was reported that the TPC of the samples increased by the increase of glycerol level in the solvent mixture (Philippi et al., 2016). Similar results for the effectiveness of the glycerol on the increase of TPC were also reported by Blidi et al. (2015).



Fig 1. Change in total phenolic content (TPC) of the samples depending on solvent type and concentration

It was informed that the main reason behind the mechanism of the effect of higher glycerol levels on the increase of TPC was the lower dielectric constant of glycerol. Additionally, the solubility of the phenolics in the plant is affected by the hydrogen bonding and steric effect of the extraction solvent (Philippi *et al.*, 2016). Also, Apostolakis et al. (2014) reported that the glycerol in the extraction solvents could increase the recovery of the polyphenolic substances due to its ability to change the water polarity.

TFCs ranged between 1.16-14.38 mg CE/g sample (Fig. 2). The lowest TFC was determined in the extract sample obtained by sole glycerol while the highest TFC was for the sample extracted by 75% glycerol as similar to TPC of the samples. Increase of glycerol concentration also increased the TFC of the samples significantly (p<0.05). Rodino et al. (2015) informed that the TFC of aerial parts of the dandelion extracts was in the range of 5-6.5 RE/g sample as quite similar to the results in the current study. For ethanol, methanol or water, the highest TFC was for the sample extracted by 50% ethanol and it was seen that the 50% ethanol was same with 50% glycerol on the extraction ability of flavonoids.



Fig 2. Change in total flavonoid content (TFC) of the samples depending on solvent type and concentration

It could be said that the TFC results according to the solvent type were correlated well with the results of TPC positively (r=0.813). Similar results were also reported as the increase in glycerol concentration increased the TFC of the samples due to the reduced polarity, increased hydrogen bonding capacity and steric effects (Eyiz *et al.*, 2020, Philippi *et al.*, 2016).

3.2 Antiradical activity of dandelion extracts

Radical scavenging activity of the dandelion extracts obtained by different solvent was evaluated by DPPH and ABTS⁺ radical scavenging tests. It was observed that the strong antiradical activity was observed for the samples. Fig.3 shows the change in the remaining DPPH after the scavenging activity of the extract samples obtained by different solvents and as is seen clearly, a significant difference was determined in terms of antiradical performance of the samples (p<0.05). The highest remaining DPPH ratio was calculated for the solvent of sole methanol, glycerol and water which also having the lowest total flavonoid and total phenolic content compared to other solvents.



Fig 3. Change in DPPH radical scavenging activity of the samples depending on solvent type and concentration

It was concluded that the sole solvent usage had no significant effect on the extraction of the strong bioactive compounds because the lowest remaining DPPH ratio which means the strong antiradical activity was determined for the aqueous glycerol (75%). As compared to aqueous ethanol and methanol, a significantly higher DPPH radical scavenging activity was observed for the aqueous glycerol. It was calculated that there was a negative and significant correlation with the remaining DPPH and total phenolic content (r=-0.86, p<0.05) and total flavonoid content (r=-0.81, p<0.05). Similar to the DPPH radical scavenging activity, the samples also showed ABTS.+ radical scavenging performance. Fig. 4 illustrates the change in ABTS.⁺ radical scavenging activities of the samples and it is clear from the results that the change in antiradical activities of the samples showed significant differences (p<0.05).



Fig 4. Change in ABTS⁺ radical scavenging activity of the samples depending on solvent type and concentration

The lowest ABTS⁺ radical scavenging activity $(9.29 \ \mu g \ Trolox/g)$ was calculated for the solvent of sole glycerol and also methanol while the highest ABTS⁺ radical scavenging activity $(33.45 \ \mu g \ Trolox/g)$ was determined for the

aqueous glycerol (75%) as similar to the DPPH radical activity. So, the strong antiradical performance of the aqueous glycerol (75%) was validated by two common antiradical scavenging test. A significant correlation was also observed between TPC and ABTS.⁺ radical scavenging activity (r=0.97, p<0.05). Also the correlation between two studied radical scavenging test (DPPH and ABTS.⁺) was also high (r=0.91, p<0.05). Eyiz et al. (2020) reported that the glycerol increase showed a higher antiradical activity for the grape pomace extracted due to the increased glycerol provided a higher total phenolic yield because a significant and quite high correlation (r=0.869, p<0.05) was observed between TPC and antiradical activity of red grape pomace (Eviz et al. 2020). Shehata et al. (2015) also reported that the aqueous mixtures of glycerol up to 90% (w/v) showed quite high satisfactory yields for total phenolics from two Artemisia species, at the same liquid-to-solid and the glycerol levels were correlated with the antiradical activities of the samples.

3.3 Antioxidant capacity of dandelion extracts

Antioxidant performance of the samples was evaluated by ferric reducing power, ferrous ions chelating activity and phosmolybdenum antioxidant activity test procedures because the antioxidant system is quite complex and it is affected by many factors and so, one method is not enough to describe the antioxidant activity of a sample (El Jemli et al., 2016). Fig. 5 shows the ferrous ions chelating ability of the samples. As is seen, the samples showed different chelating performance and the differences among the activities were significant (p < 0.05). The highest chelating activity was for the samples of aqueous methanol, ethanol and glycerol, respectively. Sole methanol and glycerol showed the weakest chelating performance. Ferrous ions chelating activity shows the antioxidant power of the samples because it was reported that the ferrous state of iron stimulate lipid peroxidation and iron was known to be the most powerful pro-oxidant among the various species of metal ions (Salar et al., 2015).



Fig 5. Change in ferrous ions chelating activity of the samples depending on solvent type and concentration

It was observed that there was a positive correlation between the chelating performance and TPC of the samples (r=0.73, p<0.05) and the correlation was also verified by Budhiyanti *et al.* (2011) who reported that the ferrous ions chelating ability of the extracts increased by the increase of TPC. Khokhar and Apenten (2003) reported that the performance of the phenolic substances was related to the ortho-dihydroxy polyphenols. Similarly, it was also informed that the metal chelating activities of the phenolic structure and the hydroxyl group location and number (Santoso *et al.*, 2004; Andjelkovic *et al.*, 2006).

Ferric reducing antioxidant power of the samples was showed in Fig. 6. Ferric reducing activity shows the antioxidant performance of the sample extracts. As is seen, aqueous glycerol solution exhibited the strongest ferric reducing activity (31.01 mg AAE/(kg) compared to other solvents to extract the samples. In addition to that, the other aqueous solvents namely ethanol and methanol also showed a weaker antioxidant capacity at all water:alcohol ratios compared to

glycerol. As similar to ferrous ion chelating activity, the lowest ferric reducing power was measured for the sole solvents namely glycerol (6.15 mg AAE/kg), methanol (7.78 mg AAE/kg) and water (8.44 mg AAE/kg). It was calculated that there was quite high and significant correlation between ferric reducing antioxidant activity and TPC (r=0.941), TFC (r=0.821), DPPH radical scavenging activity (r=0.94) and ABTS.⁺ radical scavenging activity (r=0.983). Similar results were also reported by El Jemli et al. (2016) and they found that ferric reducing activity was directly depended on TPC and they reported that there was high correlation between ferric reducing activity and TPC (r=0.911), TFC (r=0.986), DPPH radical scavenging activity (r=0.957) and ABTS⁺ radical scavenging activity (r=0.848). Amin et al. (2013) also reported that these tests were important to show the antioxidant power of the extracts because the chelating capacity provided a reduction in the concentration of the catalyzing transition metal.



Fig 6. Change in ferrous reducing antioxidant activity of the samples depending on solvent type and concentration

Hossain *et al.* (2014) reported that the ferrous ions chelating or reducing activities of the plant extracts show their bioactive performance because iron plays a significant role in the generation of free radical substances and the extracts showing ferrous reducing or chelating performance delays the oxyradical

generation and the consequent oxidative damage.



Fig 7. Change in antioxidant capacity of the samples depending on solvent type and concentration

Antioxidant activity of the extract samples obtained by different solvents was also evaluated by phosphomlybdenum approach. Sole glycerol extracts showed the weakest antioxidant activity while the strongest antioxidant performance was also for aqueous glycerol (75%) (Fig.7).

Also the mixture of glycerol:water at 50:50 showed quite similar activity in terms of antioxidant performance of 75% glycerol solvent. Antioxidant activity of the samples was well correlated with TPC (r=0.85, p<0.05), TFC (0.93, p<0.05), ferric reducing activity (r=0.87, p<0.05) and antiradical activity (r=0.87, p<0.05).

4. Conclusions

The present study showed that the extraction of dandelion by using aqueous glycerol is quite efficient process to increase the bioactivity of the produced extracts compared to other solvents such as water, ethanol, methanol or their aqueous mixtures. Among the studied concentrations namely 25, 50, 75 and 100% for the solvents, the best effect in terms of total phenolic content, total flavonoid content, ABTS.⁺ and DPPH radical scavenging activities, and also antioxidant capacities was determined for the solvent of 75% glycerol. It was observed that the hydroglycerolic extraction is the best way to produce green extract from the medicinal plants like dandelion and the effect of aqueous glycerol should be investigated for other important plants used in phytomedicine.

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