Determination of Antioxidant Activity and Total Phenolic and Flavonoid Content of Walnut (Juglans Regia) Leaves Collected from Kayseri Region

Ismail Bahadır Erdem[1]*, Nalan Özdemir[1],

[a] *Erciyes University, Faculty of Science, Department of Chemistry, 38039, Kayseri, Turkey.*

ABSTRACT

- Since ancient times, medical plants have been used to treat various diseases[1]. The green shell, seed, kernel and leafs of walnut (*Juglans regia*) plant are used in traditional medicine for their hypoglycemic, antidiarrheal, antifungal, hypotensive, sedative, vascular strengthening, hemostatic, and anthelmintic properties[2]. As it is known, oxidative stress is the cause of many important diseases, and for this reason, the antioxidant effect of walnut plant has been examined pharmacologically in some studies[3].
- In this study, walnut leaves were collected from some walnut (*Juglans regia*) trees growing in the Kayseri region (Büyük Bürüngüz area in the Koramaz Valley, Türkiye) at different periods of the year. Then, the antioxidant activity and total phenolic content of these collected walnut leaves were determined. For this purpose, firstly, walnut leaves were collected periodically at 3-month intervals from June 2022 to November 2022. Then the leaves were dried properly. After that, dried walnut leaves were extracted successively with methanol:water (3:4) by using Soxhlet technique. The phenolic and flavonoid content of obtained extracts were determined by spectrophotometrically. The antioxidative activity was also tested by spectrophotometrically. Then, the antioxidant activity, total phenolic and flavonoid contents of the extracts obtained from periodically collected leaves were investigated and compared with those collected in other periods.

MATERIALS & METHODS

3 different types of walnut leaves which *Chandler, Franquette* and *Eureka* were collected in different periods at Büyük Bürüngüz,Kayseri region. Then all the samples were extracted with Soxhlet apparatus with metanol:water solution

Figure 1 . Methanolic exraction with soxhlet app.

The solutions were evaporated. Extracts were obtained

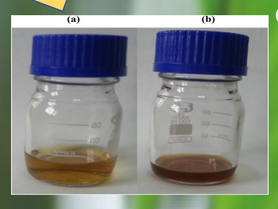

Figure 2 . Obtained extracts

Antioxidant Activity

Ascorbic acid and extract solution was preperad with the different ratios from 100 ppm to 5ppm. Also 50 ml 0,1 mM DPPH solution was Then 0,1 mM DPPH was added prepared ascorbic acid and extracts and incubated in dark area for 30 minutes. The changes in the absorbance of the samples were measured at 517 nm

Total Phenolic Content

Gallic acid solution and sample exracts were preapred and folin reagent is added. It was incubated in dark area for 5 min and lastly %20 sodium carbonate solution added. The absorbance of the samples were measured at 765 nm.

Total Flavonoid Content

Quercetin and extacts solutions were added % 10 $Al(NO_3)_3$ and 1 M KCH_3COO. After incubation for 5 minutes, Then supernatent is readed at 415 nm for each. The absorbance of the samples were measured at 415 nm.

RESULTS AND DISCUSSION

3 types of walnut leaves which *Chandler, Franquette* and *Eureka* were extracted in 3 different periods when June, September and November. According to period and type of walnut, phenolic content, flavanoid content and antioxidant activity changes were observed. Before these analysis, the obtained extracts from 1th month and 6th month. All the samples were charactirized due to essential fatty acids, phenolic and flavonoid compounds with gc-ms method. The results are shown at Table-1 and Table-2. It is indicated changing of compounds in the extract at the first and last month period. The essential fatty acid, fenolic and flavanoid contents of the extracts vary according to the results obtained in the first and last months.

different periods

	J. Regia Franquette (1. Month)	*J. Regia Franquette (6. Month)*	*J. Regia Chandler (1. Month)*	*J. Regia Chandler (6. Month)*	*J. Regia Eureka (1. Month)*	*J.Regia Eureka (6. Month)*
Undecanoic A (Area %)	**0**	0,7961	0	1,3818	0	0,6
Lauric A (Area %)	**0**	2,2656	0	1,9279	0	0,89
Tridecanoic A (Area %)	**0**	1,7951	**0**	1,3036	0	1,1152
Mystric A (Area %)	**0**	0,9452	0,3321	1,5821	0	1,2769
Palmitic A (Area %)	**26,0322**	19,1072	31,1952	21,1658	30,2917	19,7687
Heptadeconoic Acid (Area %)	**3,2614**	2,4776	2,5812	1,1593	2,4491	1,6641
Elaidic A (Area %)	**5,2956**	9,7879	5,3341	10,0727	5,476	6,3295
Oleic (Area %)	**9,401**	10,5545	7,4589	4,1509	13,307	7,6451
Linoleic A (%Area)	**11,7517**	9,1357	10,4882	6,4547	12,1704	7,5373
Cis11,14 Eicosadienoic (Area %)	41,9183	30,5691	42,6103	33,9312	32,969	37,8526
Arachidonic A (Area %)	0	1,6411	0	0,9806	0	1,2049
Lignoceric A (Area %)	2,3397	10,925	0	15,8892	3,3367	14,1157

J. Regia Franquette and J. Regia Chandler leaves have the highest total phenolic substance content in the 4th month. J. Regia Euraka leaf has the highest total phenolic substance content in the 6th month (Figure 3).

J. Regia Franquette and J. Regia Chandler leaves have the highest total flavanoid substance content in the 4th month. J. Regia Euraka leaf has the highest total flavanoid substance content in the 6th month (Figure 4).

Table 2 : Phenolic and flavanoid profiles in the extracts of J. regia leaves collected at different periods

	J. Regia Franquette (1. Month)	*J. Regia Franquette (6. Month)*	*J. Regia Chandler (1. Month)*	*J. Regia Chandler (6. Month)*	*J. Regia Eureka (1. Month)*	*J. Regia Eureka (6. Month)*
1,2,3-Propanetriol (CAS)(Area%)	17,32	2,37	14,07	2,08	6,04	4,55
1-Propylmethyl Ether (Area%)	8,85	4,27	7,43	4,38	5,65	0
Performic Acid,Trimethylsilyl Derivative (Area%)	0	0	8,87	0	2,41	0
Acetic Acid,Hydroxy-,Methyl Ester (CAS) (Area%)	5,43	0	4,74	1,66	2,69	1,36
2-Butanone,3-Hydroxy-(CAS)(Area%)	4,46	0	4	2,22	2,57	0
Propanoic Acid,2-Hydroxy-,Methyl Ester(Area%)	36,85	0	32,28	8,1	0	0
2-Pentanol,2,4-Dimethyl-(CAS)(Area%)	0	0	0,82	0	0	0
2-Cyclopenten-1-one(Area%)	3,57	4,97	2,93	1,95	2	1,83
1,3-Dioxoltane-4-Methanol,2-Etyhl(Area%)	0	0	2,63	0	0	0
Phenol,2-Methoxy-)(Area%)	0	1,79	0		0	3,42
Phenol,2-Methoxy-4-(2-Propenyl)-(CAS) (Area%)	17,45	10,87	18,53	21,51	24,78	21,54
Tetradecanal (CAS)	0	0	3,7	0	0	0
2,3-Dihydro-Benzofuran(Area%)	*0*	*17,18*	*0*	*31,98*	*17,64*	*44,04*
2-Methoxy-4-vinylphenol(Area%)	0	7,92	0	10,01	0	0
Phenol,2-Methoxy-4-(1-Propenyl)(Area%)	0	2,74	0	2,8	0	0
Benzene,(1-ethyloctyl)-(Area%)	0	0,89	0	0,79	0	0
Benzene,(1-ethylnonyl)-(Area%)	1,83	1,66	0	2,41	2,23	0
Benzene,(1-Pentylhexyl)-(Area%)	0	0	0	3,18	0	0
Benzene,(1-Butylheptyl)-(CAS)(Area%)	1,83	3,18	0	2,11	4,05	4,79
Benzene,(1-Propyınonyl)-(CAS)(Area%)	0	1,94	0	1,89	1,43	0
Benzene,(1-Propyloctyl)-(Area%)	0	0	0	0	1,35	1,84
Benzene,(Methyldecyl)-(Area%)	1,15	0	0	2,93	0	1,84
Benzene,(1-ethyldecyl)-(Area%)	0	0	0	0	1,02	0
Benzene,(1-butyloctyl)-(Area%)	0	2,65	0	0	0	2,5
(Z)6-Pentadecen-1-ol(Area%)	1,26	0	0	0	1,03	3,19
Elemol(Area%)	0	2,4	0	0	0	0
Glycerin(Area%)	0	2,94	0	0	5,54	0
2-Propanol (CAS)(Area%)	0	7,9	0	0	19,57	0
Cis-1,2-Dihydroacetechol(Area%)	0	10,43	0	0	0	0
Benzoic Acid3Methyl Ester(Area%)	0	1,66	0	0	0	0
Ethanone(Area%)	0	0,46	0	0	0	0
Beta Eudesmol(Area%)	0	10,74	0	0	0	0

J. Regia Franquette and J. Regia Chandler leaves have the highest total phenolic substance content in the 4th month. J. Regia Euraka leaf has the highest total phenolic substance content in the 6th month (Figure 5). The results obtained are quite compatible with the total phenolic and flavonoid contents of the leaves.

Ascorbic acid was used as a positive control in this study. Other results were compared with the positive control. 100 ppm plant extracts exhibited very high antioxidant activity.

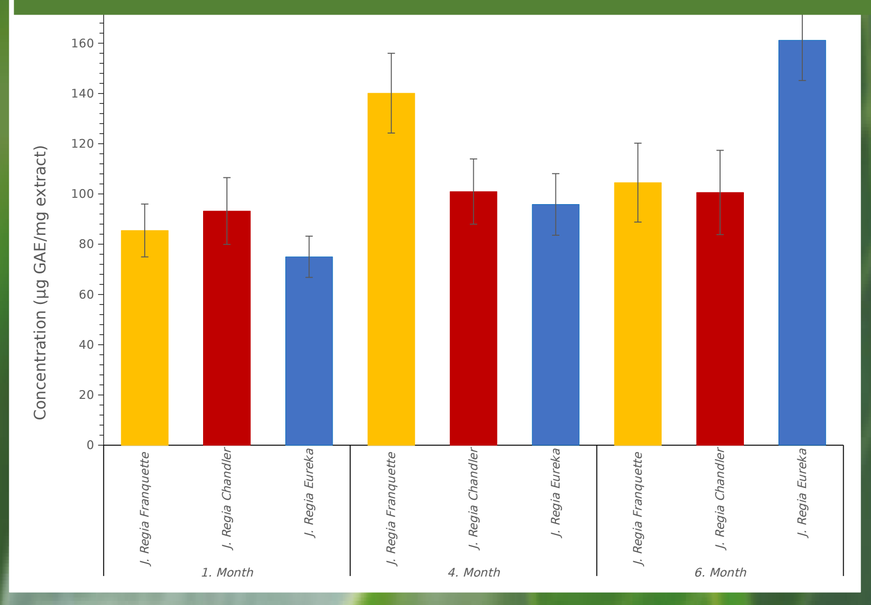

Figure 3 : The amount of total phenolic compounds equivalent to gallic acid (GAE) in the extracts of J. regia leaves collected at different periods

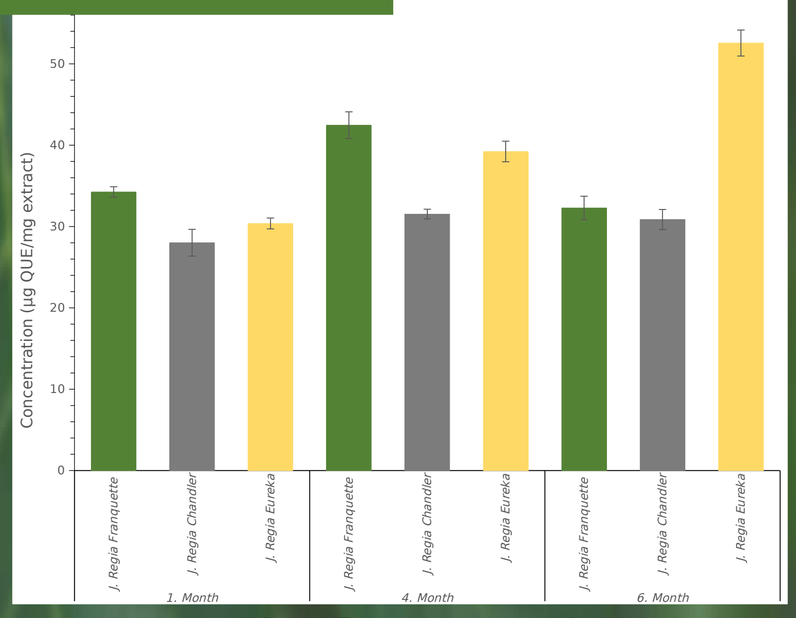

Figure 4 : The amount of total flavonoid compounds equivalent to qercetine (QUE) in the extracts of J. regia leaves collected at different periods

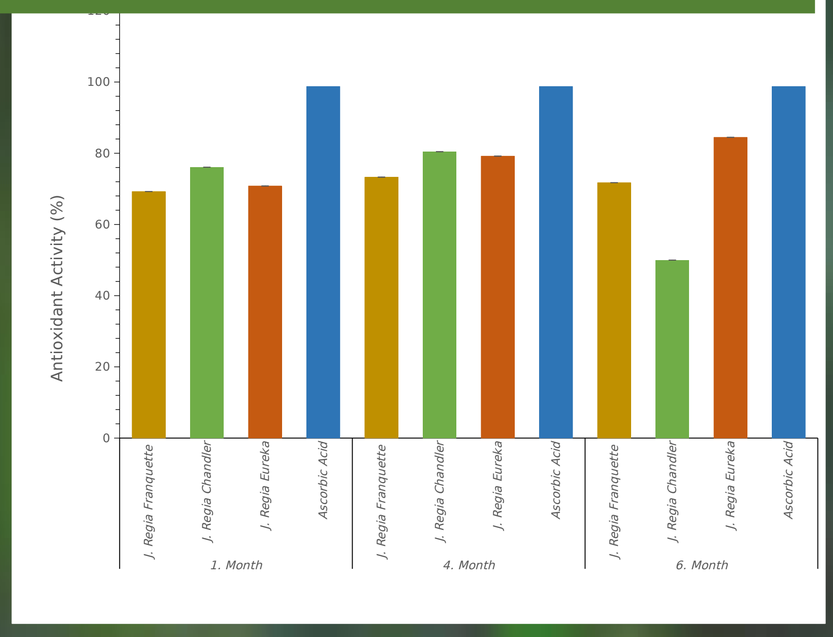

Figure 5 : Antioxidant activity of the extracts of J. regia leaves collected at different periods

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