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Chemical Composition and Biological Activity of *Lomelosia rotata* (M.Bieb.) Greuter & Burdet

Sevil Albayrak [1*], Gülsen Şimşek Gülhan [2] and Ahmet Aksoy [3]

[1]*Department of Biology, Science Faculty, Erciyes University, 38280, Kayseri, Türkiye*

[2] *Department of Biology, Graduate School of Natural and Applied Science, Erciyes University, 38280, Kayseri, Türkiye*

[3]*Department of Biology, Science Faculty, Akdeniz University, 07070, Antalya, Türkiye*

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Abstract: This study aimed to determine the phenolic compositions and biological activities of *Lomelosia rotata* (M.Bieb.) Greuter & Burdet (Caprifoliaceae). According to HPLC/MS-MS analysis results, quinic acid (253.058 mg/g) was detected as the primary component in *L. rotata* methanol extract. The total phenolic and total flavonoid contents of the extract were examined using the Folin-Ciocalteu reagent and aluminum chloride colorimetric assays, respectively. Phosphomolybdenum, 2,2-diphenyl-1-picrylhydrazyl radical-scavenging (DPPH), ferric-ion-reducing power (FRAP), and cupric-ion-reducing antioxidant activity (CUPRAC) methods were used to evaluate the antioxidant capacity. *L. rotata* methanolic extract showed an effective DPPH scavenging activity (IC_{50} = 23.30 µg/mL). Results showed that the extract had moderate reducing activity toward cupric and ferric ions. The methanol extract showed no antibacterial effect. Our study suggests that *L. rotata* extract has potential use as a natural antioxidant for food preservation and human health.

Keywords: *Lomelosia rotata*, antibacterial activity, antioxidant activity, phenolics.

1. Introduction

Oxidative stress, initiated by reactive oxygen species (ROS), such as superoxide anion, peroxyl radicals, and hydroxyl radicals, plays an important role in various degenerative diseases and in the normal aging process [1]. In addition, oxidation reactions and decomposition of oxidation products are the main causes of spoilage of various food products. Antioxidants are widely used in some food products to prevent this process [2]. Antioxidants protect fats from oxidation and are also important for human health as they prevent damage caused by biological degeneration [3]. Antioxidants are defined as molecules that eliminate, delay, or prevent oxidative damage to the target molecule [4].

Flavonoids, which are naturally occurring and widely found in plants, exhibit biological activities such as antiradical, antioxidant, and anticancer effects and have become suitable precursors in drug research [5]. Due to consumers' concerns about the toxicity of synthetic antioxidants and a preference

* Corresponding author: E-Mail: salbayrak@erciyes.edu.tr

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for natural food additives, interest in natural antioxidants for use in foods is increasing. Phytochemicals with antioxidant properties are accepted for medicines, functional foods, and cosmetics [6].

The family Caprifoliaceae, comprising woody plants in the forms of climbers, shrubs, or small trees, and rarely herbaceous plants, is represented by 41 genera and about 960 species worldwide, 12 genera and 158 species in Türkiye. *Lomelosia* (Caprifoliaceae) was described for the first time by Rafinesque (1838) in the work titled "Flora Telluriana". It includes 22 taxa (21 species), 7 of which are endemic. Based on molecular phylogenetic analyses and morphological studies, some species of the genus *Scabiosa* L. (Dipsacaceae) have been transferred to the genus *Lomelosia* Raf. genus and the Caprifoliaceae family [7]. The genus *Scabiosa* is a widely used plant due to its biological activities [8]. The *Scabiosa* genus has been reported to have anti-inflammatory, antioxidant, immune, and cardiovascular system-enhancing properties [9]. Antibacterial activities of methanol extracts from *L. minoana* subsp. *asterrusica* and *L. sphaciotica* were determined by agar diffusion method against *E. coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *K. pneumoniae*, and *Enterobacter cloacae* [10]. In our previous study, the total phenolic and total flavonoid amounts, as well as the antioxidant and antibacterial capacities of *L. pseudograminifolia*, were determined [11]. Pollen morphology of *L. rotata* was investigated using a light microscope and scanning electron microscopy [12]. According to the literature review, few studies have examined the phytochemical composition and biological activities of *Lomelosia* taxa. Thus, this work was conducted to determine the phenolic composition and biological activities of *Lomelosia rotata* (M.Bieb.) Greuter & Burdet.

2. Materials and Methods

2.1. Plant Material

Lomelosia rotata (M.Bieb.) Greuter & Burdet (Caprifoliaceae) was collected from Burdur (Bucak, Tekkeköy exit, 1077 m) in the Southern Anatolia region of Turkey in Jun 2020. The plant was collected during its flowering season. The voucher specimens have been stored at the herbarium (Voucher no: Aksoy 3129).

2.2. Extraction

The aerial parts of the dried plant were ground in an electric grinder to produce a fine powder. For the maceration technique, 5 g of powder was extracted with 100 mL of methanol for 24 hours, and repeated three times. After the contents were filtered through filter paper. The filtrate was dried in a rotary evaporator at 40-50 °C and then lyophilized. The extract was kept in the refrigerator (+ 4 °C) for further study [13].

2.3. HPLC-MS/MS Analysis

For the quantitative analysis of 53 phytochemicals in the extract, Shimadzu-Nexera model ultra-high performance liquid chromatography (UHPLC) in conjunction with a Tandem Mass Spectrometer was used. The hardware of the reverse-phase UHPLC system consists of the following parts: one autosampler (SIL-30AC model), one column oven (CTO-10ASvp model), binary pumps (LC-30AD model), and one Degasser (DGU-20A3R model). Chromatographic conditions are optimized to ensure optimum separation of 53 phytochemicals. Different columns like Agilent Poroshell 120 EC-C18 model (150 mm×2.1 mm, 2.7 µm) and RP-C18 Inertsil ODS-4 (100 mm×2.1 mm, 2 µm), different mobile phases such as acetonitrile and methanol (B), different mobile phase additives such as ammonium formate, formic acid, ammonium acetate and acetic acid, different column temperatures, such as 25 °C, 30 °C, 35 °C and 40 °C tested until optimum conditions are achieved. Chromatographic separation was performed in reverse phase using an Agilent Poroshell 120 model EC-C18 (150 mm×2.1 mm, 2.7 µm) analytical column. The column temperature was set to 40 °C. The separation gradient consists of solvent A (water + 5 mM ammonium formate + 0.1 % formic acid) and solvent B (methanol + 5 mM ammonium formate + 0.1 % formic acid). % 20-100 B (0-25 dk), %100 B (25-35 dk), %20 B (35-45 dk) solvent profile is used. Solvent flow rate and injection volume were set at 0.5 mL/min and 5 µL, respectively.

Mass spectrophotometer detection was performed using a Shimadzu LCMS-8040 model tandem mass spectrometer equipped with Electrospray ionization (ESI), measuring in negative and positive ionization modes. LC-ESI-MS/MS data were obtained with LabSolutions software (Shimadzu). The

MRM (multiple reaction monitoring) mode was used for quantitative analysis. The MRM method selectively optimizes and detects the amount of phytochemicals based on screening of specialized precursor phytochemical-fragment ion transitions. Collision energies (CE) are optimized to produce optimal phytochemical fragmentation and maximum transient of desired product ions. MS conditions are as follows: drying gas (N_2) flow, 15 L/dk; nebulizing gas (N_2) flow, 3 L/dk; DL temperature, 250 °C; heat block temperature, 400 °C; and interface temperature, 350 °C [14].

2.4. Total Phenolic and Flavonoid Contents

The Folin-Ciocalteu colorimetric assay was applied to examine the total phenolic content of the extract. Briefly, 40 µL of the methanol extract solution was mixed with 2.4 mL of distilled water. 200 µL of Folin-Ciocalteau reagent was added, and the contents of the flask were mixed thoroughly. Then, 600 µL of sodium carbonate (20% Na_2CO_3) was added, and the volume was made up to 4.0 mL with distilled water. After a 2 h incubation at room temperature, the absorbance was measured at 765 nm using a spectrophotometer and compared with a gallic acid calibration curve. The data are presented as the average of triplicate analyses. Results were expressed as mg of gallic acid (GAE) equivalents/g extract [15]. The $AlCl_3$ colorimetric method was used to determine the total flavonoid content of the extract. The extract (0.5 mL) in methanol was separately mixed with 0.1 mL of 10 % aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. It remained at room temperature for 30 min, and the absorbance of the reaction mixture was measured at 415 nm. The result is reported as mean values expressed as mg of quercetin equivalent (QE)/g of dry extract [15].

2.5. Antioxidant Activity Methods

2.5.1. Phosphomolybdenum Assay

The total antioxidant activity of the methanolic extract was determined by the phosphomolybdenum method [16]. 0.4 mL of the methanolic extract was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the complex was measured at 695 nm. For the blank, methanol was used in place of the sample. Total antioxidant activity was expressed as ascorbic acid equivalents (AAE)/g extract.

2.5.2. DPPH Radical Scavenging Activity

The ability of the extract to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed spectrophotometrically [16]. 50 µL aliquots of the appropriate extract dilution, in the concentration range 0.1-2 mg/mL, were mixed with 1 mL of the methanolic DPPH solution (0.1 mM). Methanol was used as a control instead of the extract. The mixtures were left in the dark at room temperature for 30 min, and the absorbance at 517 nm was measured. The IC_{50} (concentration causing 50% inhibition) value of the extract was determined graphically. The same procedure was repeated with BHT (butylated hydroxy toluene) as a positive control. The measurements were performed in triplicate, and the results were averaged. Radical scavenging activity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation:

$$\%\text{Inhibition}=\frac{\left(\text{Abs}_{\text{control}}-\text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{control}}}\times 100$$

2.5.3. Ferric Reducing Antioxidant Power Assay (FRAP)

The ferric ions reducing capacity was determined using the Ferric Reducing Antioxidant Power (FRAP) assay [16]. The FRAP reagent was prepared by mixing an equivalent volume of 300 mM acetate buffer (pH 3.6) and 20 mM $FeCl_3$. $6H_2O$ with 10 mM TPTZ in 40 mM HCl. Briefly, an aliquot of the extract was added to a diluted FRAP reagent, incubated at 37 °C for 30 min, and the absorbance was read at 595 nm. The quantitative analysis was done using the external standard method (ferrous sulphate, 0.1-2 mmol), correlating the absorbance (λ = 593 nm) with the concentration. L-ascorbic acid was used as the positive control. The results were expressed as mM of Fe^{2+}.

2.5.4. Cupric Ion Reducing Antioxidant Capacity Assay (CUPRAC)

One mL each of 10 mM $CuCl_2$, 7.5 mM neocuproine, and NH_4Ac buffer (1 M, pH 7.0) solutions was added to a test tube. Then, 0.5 mL of the extract at different concentrations was mixed, and the total volume was brought to 4.1 mL with H_2O. The mixture absorbance was recorded against a blank at 450 nm after 30 min incubation at room temperature. Trolox was used as the positive control [16].

2.6. Antibacterial Activity Method

Test bacteria strains used in the study were *Aeromonas hydrophila* ATCC 7965, *Salmonella typhimurium* NRRLE 4463, *Listeria monocytogenes* 1/2B, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 25933, *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa* ATCC 27853, Methicillin resistance *Staphylococcus aureus* ATCC 43300, Methicillin-sensitive *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 10015, *Salmonella enteritidis* ATCC 13076, and *Mycobacterium smegmatis* RUT. Antibacterial activity test were assessed using the agar diffusion assay [17]. The density of bacterial cultures was adjusted to 10^6-10^7 cfu/mL with sterile nutrient broth. The mediums containing each bacterial culture were poured into petri dishes (9 cm). After cooling the inoculated agar, holes (6 mm) were made with stainless steel cylinders. 50 µL of the extract (60 mg/mL) and Tetracycline (10 mg/mL) were dropped into each hole. Absolute methanol without herb extract was used as a negative control. The plates were incubated at 37 °C for 24 hours. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in duplicate, and the results were presented as averages.

3. Results and Discussion

3.1. Yields, Total Phenolic and Flavonoid Contents

The percent yield of the methanol extract of *L. rotata* was 12.75% (w/w) (Table 1). The total phenolic content of the *L. rotata* methanol extract was determined using the Folin-Ciocalteu method and expressed as gallic acid equivalents (Table 1). The total phenolic and flavonoid contents were measured as 35.91 ± 0.0 mg GAE/g and 11.13 ± 0.1 mg QE/g extract, respectively (Table 1).

Table 1. The total phenolic content, total flavonoid content, and antioxidant capacities of *L. rotata*.

Parameters	Methanol Extract	BHT	L-Ascorbic acid
Yield (%)	12.75		
Total phenolic content (mg GAE/g extract)	35.91 ± 0.0*		
Total flavonoid content (mg QE/g extract)	11.13 ± 0.1		
Total antioxidant activity (mg AAE/g extract)	103.14 ± 0.5		
DPPH IC_{50} (µg/mL)	23.20 ± 0.0	6.81 ± 0.0	
FRAP mM Fe (II)	2.49 ± 0.0		4.04 ± 0.0

*Values expressed are mean ± standard deviation of three experiments. Total phenolic content is expressed as gallic acid equivalent (GAE), total flavonoid content is expressed as quercetin equivalent (QE), and total antioxidant activity is expressed as ascorbic acid equivalent (AAE).

3.2. Phenolic Compounds

The existence of quinic acid (253.058 mg/g), gallic acid (0.297 mg/g), protocatechuic acid (3.038 mg/g), chlorogenic acid (32.028 mg/g), protocatechuic aldehyde (0.3 mg/g), caffeic acid (5.229 mg/g), *p*-coumaric acid (0.233 mg/g), salicylic acid (0.046 mg/g), cynaroside (0.269 mg/g), rutin (0.456 mg/g), isoquercitrin (0.613 mg/g), hesperidin (0.679 mg/g), cosmosiin (0.19 mg/g), astragalin (0.354 mg/g), nicotiflorin (1.571 mg/g), naringenin (0.005 mg/g), luteolin (0.139 mg/g), apigenin (0.026 mg/g) and acacetin (0.004 mg/g) in the methanolic extract were detected with UHPLC-MS/MS (Table 2). Fumaric acid, aconitic acid, epigallocatechin, catechin, gentisic acid, tannic acid, epigallocatechin gallate, 1,5-dicaffeoylquinic acid, 4-OH-benzoic acid, epicatechin, vanillic acid, syringic acid, vanillin, syringic aldehyde, daidzin, epicatechin gallate, piceid, ferulic acid, sinapic acid, coumarin, miquelianin, *O*-coumaric acid, genistin, rosmarinic acid, ellagic acid, quercitrin, fisetin, daidzein, quercetin, hesperetin, genistein, kaempferol, amentoflavone and chrysin were not identified in the methanol extract. Among

the identified compounds, the highest amount was quinic acid (253.058 mg/g), while the lowest amount was acacetin (0.004 mg/g) (Table 2).

Table 2. Quantification of organic acids and phenolic compounds of *L. rotata* methanol extract*.

Compounds	mg/g extract	Compounds	mg /g extract	Compounds	mg/g extract
Quinic acid	253.1	*p*-Coumaric acid	0.233	Cosmosiin	0.190
Gallic acid	0.297	Salicylic acid	0.046	Astragalin	0.354
Protocatechuic acid	3.038	Cynaroside	0.269	Nicotiflorin	1.571
Chlorogenic acid	32.03	Rutin	0.456	Naringenin	0.005
Protocatechuic aldehyde	0.300	Isoquercitrin	0.613	Luteolin	0.139
Caffeic acid	5.229	Hesperidin	0.679	Apigenin	0.026
Acacetin	0.004				

*The LC-MS/MS validations of the standard compounds and method used herein were performed previously [14]

As far as we know, the phenolic composition, antioxidant, and antibacterial activity of the methanol extract of *L. rotata* aerial parts were reported here for the first time. However, similar results have been reported for *Scabiosa* and *Lomelosia* species in only a few studies. Phytochemical screening of many *Scabiosa* species has shown that plants belonging to this genus include triterpenes, saponins, iridoids, monoterpenoid glucoindole alkaloids, coumarins, and flavonoids [18–22]. Previous phytochemical research on *S. stellata* has shown the presence of fatty acids, β-sitosterol, stigmasterol, oleanolic and ursolic acids, bis-iridoids, and flavonoids [23, 24]. Gallic acid, catechin, chlorogenic acid, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, and caffeic acid were determined in methanol extracts obtained from leaves and flowers of *S. columbaria* subsp. *columbaria* var. *columbaria* and chlorogenic acid were found to be the major compounds [25]. The main phenolic compounds in ethyl acetate (SREA) sub-extracts from the fruits of *Scabiosa rotata* were chlorogenic acid, hesperidin, quinic acid, and isoorientin [26].

3.3. Antioxidant Activity

The total antioxidant activity of the extract was 103.14 ± 0.5 mg AAE/g dry extract in the phosphomolybdenum assay (Table 1). The effects of antioxidants in the DPPH radical scavenging assay show the hydrogen-donating capacity of a compound. DPPH radical scavenging activity was expressed as the percentage inhibition of the initial DPPH absorption by the extract. Figure 1 shows a decrease in the concentration of DPPH radical due to the scavenging ability of the methanol extract and BHT. The methanol extract exerted strong free radical scavenging activity at 0.1-2.0 mg/mL. The inhibition values of the extract were 8.04%, 19.35%, 37.95%, 68.44%, and 75.00% at 0.1, 0.25, 0.5, 1.0, and 2.0 mg/mL, respectively. The free radical scavenging activity of the extract was lower than that of the BHT (81.96 % at 2.0 mg/mL). A lower IC_{50} value indicates greater antioxidant activity. The IC_{50} value of the extract was 23.20 µg/mL. This value is very low compared to that of BHT (6.81 µg/mL) (Table 1).

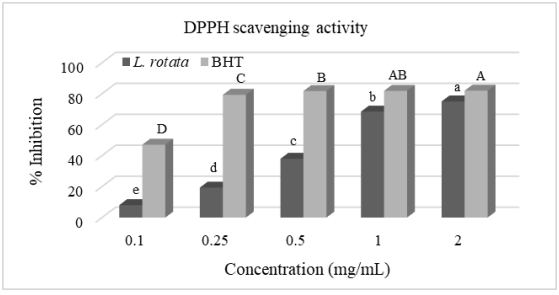

Figure 1. %Inhibition values of *L. rotata* extract by DPPH assay. (Uppercase letters represent the statistical differences between the activity of BHT; lowercase letters represent the statistical differences between the activity of the methanol extract at the different concentrations (p<0.05). BHT: butylated hydroxytoluene.)

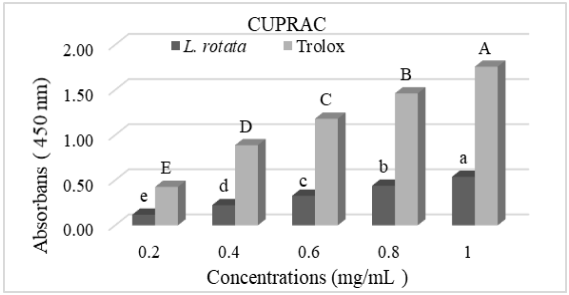

Figure 2. Antioxidant capacity of *L. rotata* extract by the CUPRAC assay. (Uppercase letters represent the statistical differences between the activity of Trolox; lowercase letters represent statistical differences between the activity of the methanol extract at the different concentrations (p< 0.05).

The cupric ion (Cu^{2+}) reducing ability of the extract is shown in Figure 2. The Cu^{2+} reducing capability measured by this method increased with concentration. Absorbance values of the extract were 0.12, 0.22, 0.33, 0.44, and 0.54 at 0.2, 0.4, 0.6, 0.8, and 1 mg/mL concentrations. These values for trolox were 0.43, 0.89, 1.18, 1.46, and 1.76 at the same concentrations. The value of the extract (0.54) was much lower than that of the positive control, trolox (1.76), at the highest concentration.

In the FRAP assay, the plant extract's ability to reduce ferric ions was determined. FRAP assay is easily reproducible and linearly related to the molar concentration of the antioxidant present [27]. The methanol extract showed lower reducing activity (2.49 mM) than L-ascorbic acid (4.04 mM) at 2 mg/mL (Table 1).

Five phenolic compounds, such as caffeic acid and luteolin glucosides, and one triterpene were isolated from *S. sicula* dichloromethane extract. In addition, the IC_{50} value of methanol extract from *S. sicula* was reported as 3.34 ± 0.01 µg/mL in DPPH assay [28]. Similarly, 34 compounds, including phenolic compounds and triterpene saponins, were identified from the methanol extract of *S. sicula*. Also, its total phenolic content and IC_{50} value in DPPH assay were found as 2.67 ± 0.01 mg GAE/g and 13.60 ± 0.01 µg/mL, respectively [29]. According to the results, the total phenolic content of the methanol extract of *L. rotata* was higher. At the same time, the radical scavenging capacity was lower than that of the methanol extract of S. *sicula*. In a previous study, antioxidant activities of ethanol extract (SRE) and its hexane (SRH), chloroform (SRC), ethyl acetate (SREA), and aqueous ethanol (SRAE) sub-extracts obtained from the fruits of *Scabiosa rotata* were studied. SREA showed the best antioxidant activity, with IC_{50} values of 54.20 µg/mL for DPPH, and also had high total phenolic (499.06 mg GA/g) and flavonoid (327.45 mg QE/g) contents [26].

3.4. Antibacterial activity

No activity against bacteria tested was shown by the *L. rotata* methanol extract at the tested concentration. Similarly, none of the extracts obtained from *Scabiosa rotata* fruits showed antibacterial activity [26]. In a previous study, the total phenolic and flavonoid contents, total antioxidant activity of the methanol extract of *L. pseudograminifolia* were determined as 50.61 mg GAE/g, 7.64 mg QE/g, and 132.53 ± 0.1 mg AAE/g, respectively. In the DPPH assay, the IC_{50} value of the extract was 41.59 µg/mL. Also, the extract showed ferric (2.87 mM) and cupric-ion reducing activity but exhibited no antibacterial activity [11].

In our previous paper, extracts from 10 different *Scabiosa* species from Türkiye were found to contain mainly chlorogenic acid (845.839 mg/g), quinic acid (751.277 mg/g), and cynaroside (629.344 mg/g). Their total phenolic and flavonoid contents ranged from 39.62-18.66 mg GAE/g and 18.45-7.51mg QE/g, respectively. Their total antioxidant activities ranged from 47.20 to 122.55 mg AAE/g. IC_{50} values of the extracts ranged from 36.88 to 22.98 µg/mL in the DPPH assay. In the FRAP assay, their iron ion-reducing activity ranged from 3.47 mM to 2.17 mM, and they showed concentration-dependent cupric ion-reducing activity. Similarly, the extracts showed only low antibacterial activity against *K. pneumoniae*, *S. pneumoniae*, and *P. aeruginosa* among the 13 tested bacteria [15]. The methanol extracts from *L. minoana* subsp. *asterusica* and *L. sphaciotica* showed antibacterial effect against *E. coli*, *S. aureus*, *Staphylococcus epidermidis* [10].

4. Conclusion

Results from various antioxidant activity assays indicate that the methanol extract of *L. rotata* has significant antioxidant activity. The present study also showed that the methanol extract of *L. rotata* had high levels of phenolics and flavonoids. The methanol extract had no antibacterial activity. As far as we know, the phenolic composition and biological activities of the methanol extract of *L. rotata* have been reported here for the first time. The methanol extract could serve as a potential source of natural antioxidant agents in the pharmaceutical and food industries.

Conflict of Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

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ORCID
Sevil Albayrak: 0000-0002-0253-3746
Gülsen Şimşek Gülhan: 0009-0002-5011-8843
Ahmet Aksoy: 0000-0002-9696-7122

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