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Determination of Phytochemical Profile, Antiquorum Sensing and Antioxidant Activities of *Tragopogon oligolepis*

Tragopogon oligolepis'ın Fitokimyasal İçeriği, Antioksidan ve Antiquorum Sensing Özelliğinin Belirlenmesi

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ABSTRACT

Objective: In this study it was aimed to examine antiquorum sensing, antioxidant activities by using root and aerial parts extracts of *Tragopogon oligolepis.* Also phenolic content was detected using HPLC analysis.

Material-Method: Antioxidant activity was detected by DPPH, FRAP methods and phenolic content HPLC. Antiquorum sensing activity was investigated by using pyocyanin and swarming motility assay on *Pseudomonas aeruginosa* PAO1.

Results: Phytochemical profile findings showed that 12 components were detected in the root and 10 components in the aerial parts. The main components were found chlorogenic acid and *o*-coumaric acid. According to the obtained of antioxidant levels the aerial parts extracts of *T. oligolepis* had the best antioxidant property in our results. The amount of DPPH (0.60 ± 0.01 mg/ml) and phenolic content (6.55±0.18 mg GAE/g sample) was determined to be high in the aerial parts. In the FRAP analysis, high reducing power was found in the roots (12.62±0.36 µmol $FeSO_4$/g sample). According to these results, although *T. oligolepis* extracts do not reach very high amounts in terms of antioxidant results, it is thought to be a plant that can be evaluated in terms of removing oxidant effects. The results of antiquorum sensing activity showed that both root and aerial parts extract showed strong inhibitory effect on swarming motility 62%, %65 rate respectively.

Conclusions: *Tragopogon oligolepis*, an endemic species, can be evaluated as an antiquorum sensing inhibitor candidate with its phytochemical contents.

Keywords: DPPH, FRAP, Pseudomonas, *T. oligolepis*

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ÖZET

Amaç: Bu çalışmada, *Tragopogon oligolepis*'in kök ve toprak üstü kısım ekstraktları kullanılarak antiquorum sensing ve antioksidan aktivitelerinin incelenmesi amaçlanmıştır. Ayrıca HPLC analizi kullanılarak fenolik içerik tespit edilmiştir.

Gereç Yöntem: Antioksidan aktivite DPPH, FRAP yöntemleri ve fenolik içerik HPLC ile tespit edildi. Çevreyi algılama aktivitesi, *Pseudomonas aeruginosa* PAO1 üzerinde piyosiyanin ve kayma hareketi testi kullanılarak araştırıldı.

Bulgular: Fitokimyasal profil bulguları, kökte 12 bileşen ve toprak üstü kısımlarda 10 bileşen tespit edildiğini göstermiştir. Ana bileşenler klorojenik asit ve o-kumarik asit olarak bulunmuştur. Elde edilen antioksidan seviyelerine göre, sonuçlarımızda en iyi antioksidan özelliği *T. oligolepis*'in toprak üstü kısımları ekstreleri göstermiştir. Toprak üstü kısımlarda DPPH (0.60 ± 0.01 mg/mL) ve fenolik içerik (6.55±0.18 mg GAE/g numune) miktarının yüksek olduğu belirlendi. FRAP analizinde köklerde yüksek indirgeme tespit edildi (12.62±0.36 µmol FeSO4/g numune). Antiquorum sensing sonuçlarına göre ise kök ve toprak üstü ektraktları kayma hareketi üzerine %62 ve %65 oranında güçlü inhibisyon etki göstermiştir.

Sonuç: Elde edilen sonuçlar neticesinde endemik bir tür olan *T. oligolepis*, fitokimyasal içeriği ile antiquorum sensing inhibitör adayı olarak değerlendirilebilir.

Anahtar Kelimeler: DPPH, FRAP, Pseudomonas, *T. oligolepis*

1. Introduction

The genus *Tragopogon L*. (*Asteraceae*) contains about 150 species worldwide and widespread in semiarid and mountainous regions of Europe and Asia [1]. 26 taxa belonging to 22 species are distributed throughout Turkey [2]. It is morphologically similar to *T. oligolepis* Hartvig & Strid is local endemic to the Southwest of Anatolia [3]. It is a perennial herb with glabrous (4-5 mm diameter) stem and vertical woody stock [4]. Plants are important as they are sources of bioactive compounds. Phenols include a large group of compounds that contribute majorly to the human diet. Long-term consumption of polyphenol-based diets has positive antioxidant effects on the human body, reducing the risk of cancer, cardiovascular diseases and diabetes. The most important polyphenol compounds are classified in phenolic acids, flavonoids, stilbenes and lignans [5].

In all parts of the world, especially in developing countries, diseases caused by fungi, viruses, bacteria, and parasites are a major cause of mortality and morbidity [6]. Undoubtedly, one of the most important discoveries for humanity in the last century is antibiotics but misuse is one of the biggest problems of recent years also [7]. The considerable time it takes to produce new antibiotics has necessitated the development of multiple strategies in the fight against infectious diseases [8]. One of the two strategies that has attracted the most interest of researchers is plants and the other is inhibition of the QS mechanism. In recent years, researches on the antimicrobial properties of phytochemicals studies on the mechanisms of action are progressing rapidly [9]. Quorum sensing system is the communication mechanism between bacteria, which occurs as a result of the expression of some genes, depending on the cell population density, through a some small molecules called AHL and expression of many virulence factors in *P. aeruginosa* occurs through this system [10]. For this reason, inhibition of virulence factors produced by bacteria in order to cause infectious disease in the host is considered as one of the important target points that can be used in the fight against bacteria.

Since there is limited study about the properties of the *T. oligolepis*, the aim of the current study was to determine the antimicrobial and antiquorum sensing activity, chemical compounds of its fraction (using HPLC method) antioxidant properties (total phenols, radical scavenging activity by IC_{50} of DPPH test and total antioxidant capacity) of its extract.

The *T. oligolepis* extract was not previously studied in terms of polyphenol content and antioxidant activity, therefore the purpose of our study was to evaluate the antioxidant effect for future studies.

2. Material and Method

Plant Material and Preparation of the Extract for Assays

The *T. oligolepis* plant used in this study was collected in Muğla, Turkey, in August 2017 and identified by Prof. Dr. Kamil Coşkun Çelebi. The plant has been stored in the Herbarium of the Department of Biology of Karadeniz Technical University (KTUB) with the herbarium number Coşkunçelebi & Gültepe 584a. The assembled and exsiccated plant samples were separated from the aerial parts and roots and ground with the help of a steel blender (Waring 8011 EB, USA). The solvent (1/10 ethanol) was added and then the solvent-sample mixture was kept in an ultrasonic bath for 30 minutes, it was filtered with coarse filter paper and the solvent was removed in a rotary evaporator (Heidolph Hei-Vap Rotary Evaporator) at 40-45ºC under vacuum. The plant extracts remaining in the balloon were weighed and recorded and taken with DMSO. The final concentration of the extract to be used in antiquorum sensing activity experiments was set as 100 mg/mL for aerial parts and root. For antioxidant analyzes spectrophotometric techniques were used and also total flavonoids, polyphenols. These techniques are constantly utilization for the native substances. Determination of antioxidant assay; powdered aerial parts (5g) and roots (5g) were separately extracted with 90-96% methanol. These extracts mixed-incubated for 24 h at low rpm and room temperature. The methanolic extracts were filtered with filter papers and used. Analyses were done three times.

Total Phenolic Assay

According to the Folin-Ciocalteu method the amount in the samples was determined [9]. Gallic acid was used as standard. The solutions absorbances of were determined for 760 nm. Concentrations of total phenolic compounds were determined for dry weight of sample as mg of gallic acid.

The Determination of Antioxidant Activity

Utilizing by FRAP technique were calculated for plants antioxidant contents. FRAP test was utilized calculated antioxidant activity. This technique is based on the reduction of Fe^{3+}-TPTZ compound to Fe^{2+} -TPTZ compound with electron donating material [10]. The result as µmol $FeSO_4.7H_2O$ was explained for dry sample.

Opposite DPPH radical the radical cleaning capacity of samples in spectrophometer was defined on 517 nm. The color change of the DPPH mixture is examined during the analysis. DPPH radical is deactivated in the presence of antioxidants [11]. The radical deactivation property was determined using Trolox and the results were expressed as IC_{50}.

Antiquorum Sensing Assay

The inhibition effect of the plant extract on the swarming motility of PAO1 was carried out by preparing a medium containing nutrient broth, noble agar and 0.5% glucose. A two hundred µL of the plant extract were added to 20 mL of the swarming medium. An overnight culture of PAO1 was centrifuged into the center of the solidified medium, and 5 µL of the supernatant was inoculated and incubated overnight at 37°C. The sliding motion at the end of incubation was evaluated by measuring the diameter of the motility from the center of inoculation to the edge. The results were evaluated by comparing it with the positive control PAO1 [14].

For pyocyanin assay; an overnight bacterial culture calculated at a density of 0.02 at OD 600 was added to 10 mL of LBB medium added with 400 µL of plant extract and incubated for 16-18 hours at 37ºC in a shaking incubator. After the incubation period, 5 mL of chloroform was added to the culture and vortexed for 30 seconds. The sub-phase formed in the medium and separated from the

chloroform was transferred to 2 mL tubes. One mL of HCl-water mixture (0.2 mol/L HCl) was added and vortexed again for 30 seconds. The absorbance of the pink phase formed at the top of the tubes was measured at 520 nm. PAO1, without added extract, was used as a positive control [15].

Statistical Analysis

Data analysis was carried out using Microsoft Office Excel 2016. For each sample, experiments were repeated three times and the mean of the results was calculated. Antiquorum sensing experiments were carried out in triplicate according to the randomized plot design and the data obtained were subjected to variance analysis using the JMP 8 packet statistics program. Statistical differences were marked by the LSD multiple comparison test.

3. Results

The antioxidants in plants are very different from each other and it is very difficult to measure each antioxidant component respectively. FRAP and DPPH methods were used in this study for radical scavenging activity. Total phenolic and flavonoid concentrations were also calculated.

Using The Folin-Ciocalteu assay the phenolic contents of the extracts measured by ranged from 5.166±0.18 to 6.55±0.18 mg GAE/g dry extract. It was found that the highest phenolic content was obtained in the aerial parts (Table 1).

FRAP and the total phenolic content values of the aerial and root parts are shown in Table (1). Analysis of DPPH values determined as the IC_{50} are shown in Fıgure (1).

Table 1: Total Phenolic Contents and FRAP for plant extracts

Analyses	Aerial parts	Roots
Total Phenolic Contents (mg GAE/g sample)	6.55±0.18	5.166±0.18
FRAP (µmol $FeSO_4$/g sample)	4.98±0.17	12.62±0.36

The IC_{50} of DPPH assay in the examined extracts ranged from 0.60 to 1.83 mg/mL. The stable free radical DPPH has been widely used in the assessment of radical scavenging activity of plant excracts, natural compounds and foods [16].

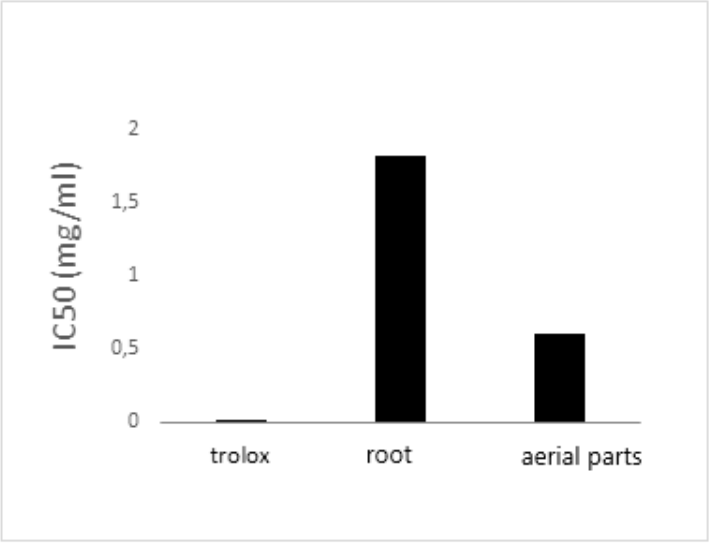

Fıgure 1: The results of DPPH for plant extracts

Bioactive Compounds

The bioactive contents of the extracts prepared with the root and aerial parts of *T. oligolepis* were studied in HPLC and a total of 23 standards were scanned. According to the results obtained, it was seen that the aerial part had more content in total in the investigated standards. Chlorogenic acid the major component in both extracts was determined, while the value found at 814.86 µg/g in the aerial parts was determined as 323.1 µg/g in the root (Table 2, 3).

Table 2: Phenolic composition of *T. oligolepis* aerial parts extract

Compounds	µg/g	Compounds	µg/g
Phenolics acids		***Flavonoids***	
chlorogenic acid	814,86	rutin	*
o-coumaric acid	157,86	catechin	*
caffeic acid	32,43	hesperidin	7,79
gallic acid	8,96	epicatechin	*
syringic acid	12,89	quercetin	*
p-coumaric acid	7,21	luteolin	32,46
protocatechic acid	*	kamferol	27,79
p-hydroxy benzoic acid	*	apigenin	92
vanillin	*		
ferulic acid	*		
sinapinic acid	*		
benzoic acid	*		
rosmarinic acid	*		
cinnamic acid	*		

** Not detected*

Table 3: Phenolic composition of *T. oligolepis* root extract

Compounds	µg/g	Compounds	µg/g
Phenolics acids		***Flavonoids***	
chlorogenic acid	323,1	rutin	*
o-coumaric acid	52,7		
caffeic acid	19,4		
gallic acid	*		
syringic acid	3,8	catechin	5,3
p-coumaric acid	0,4		
protocatechic acid	1,0		
p-hydroxy benzoic acid	2,1	hesperidin	
Vanillin	0,9		
ferulic acid	*	epicatechin	*
sinapinic acid	1,6	eriodictiol	*
benzoic acid	3,8	quercetin	*
rosmarinic acid	*	luteolin	*
cinnamic acid	0,7	kamferol	*
		apigenin	*

* Not detected

Antiquorum Sensing Activity Results

T. oligolepis methanol extract showed similar results to the swarming motility and pyocyanin pigment production, which play an important role in the virulence of *Pseudomonas*. While the inhibition rates of the root extract were 61% on the swarming, it was determined as 33% on the pyocyanin pigment production. On the other hand the aerial parts extract showed a strong inhibition effect of 65% on the swarming motility, while a low inhibition effect of 15% on pyocyanin production.

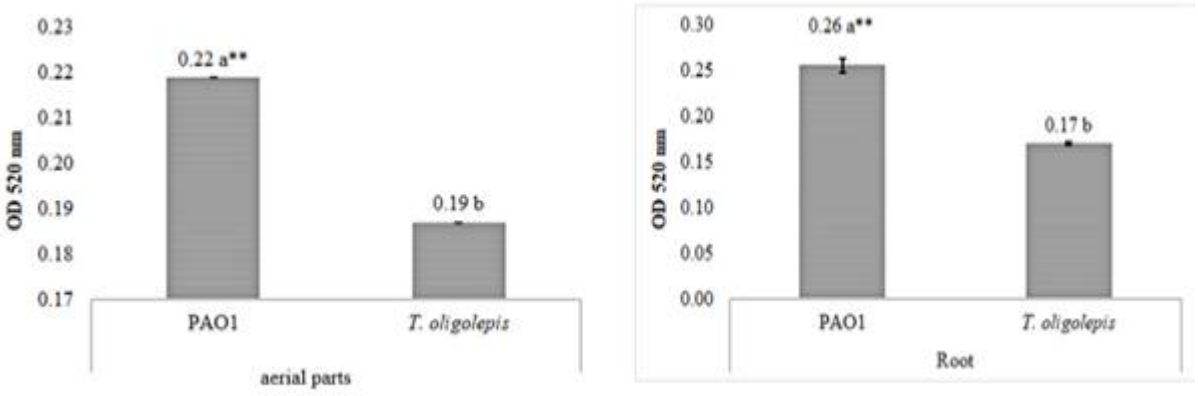

Figure 2. Inhibition effect of extracts on pyocyanin production. **Differences between mean values followed by different letters of compounds are statistically significant at $p < 0.01$

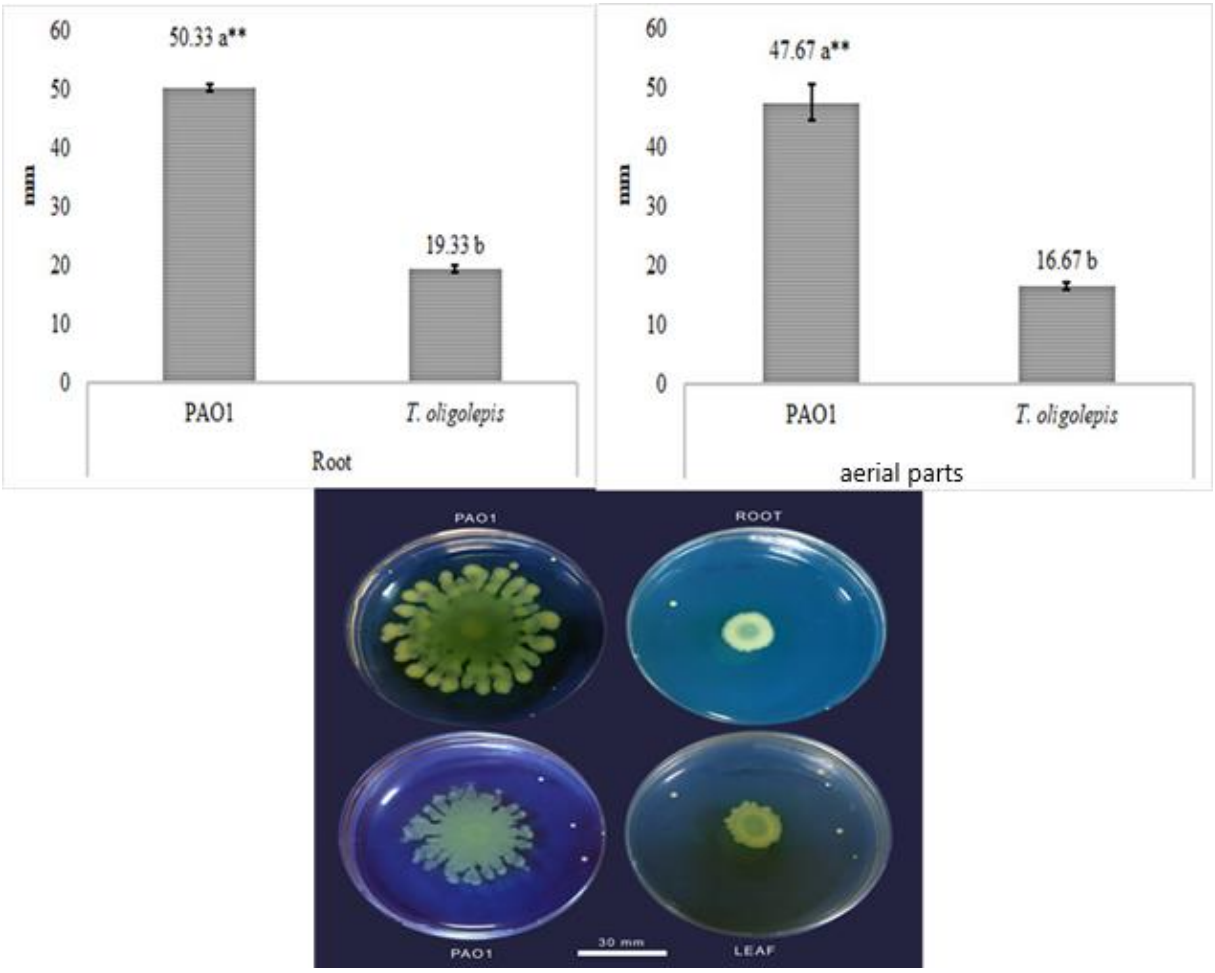

Fıgure 3. Inhibition effect of extracts on swarming motility, (scala bar 30mm). **Differences between mean values followed by different letters of compounds are statistically significant at $p < 0.01$

4. Discussion and Conclusion

Plants with medicinal properties and their secondary metabolites have been discovered since the dawn of time. Depending on the type of plant, the use of flowers, leaves, branches or roots is common in the treatment of acute and chronic diseases. Especially important to limit oxidative reactions in cells antioxidant nutrients are predispose humans to the development of major clinical conditions. Today, such as flavonoids, the antioxidant potential of plant-derived phenolic compounds are great interest in the possibility that may reduce the risk of developing these conditions [17]. Phenolic compounds may have a direct contribution to have an antioxidant effect [18]. Flavonoids are well known antioxidants. In different studies, antioxidant activities of flavonoid-rich plant extracts were found to be quite high [16,19]. Farzaei M.H. et al. identified the chemical constituents of the essantial oil from the aerial parts of *Tragopogon graminifolius* by GLC and GLC-MS [20].]. In our study, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, and o-coumaric acid were found as common components in both samples. Chemical components of Hexane extract of *T. oligolepis* obtained from Muğla region (Turkey) were investigated by Uğur A. et al. who studied the same *Tragopogon* species as us, with GLC-GLC-MS the main components of the hexane extract were characterized as caryophyllene oxide (18.5%)[21]. Chlorogenic acid was found to be 323.1 µg for root extract and 814.8 µg for aerial parts extract in our study. Uysal S. et al. [22] indicated the presence of chlorogenic acid in water and methanolic extracts of *Tragopogon dubius* obtained from Kastamonu, Turkey. The authors reported that the highest content of phenolics was detected in the methanolic extracts. In addition, the authors stated that chlorogenic acid was the dominant natural product and benzoic acid observed in the methanolic extract, as we determined in our study. According to Sareedenchai V. et al. and Granica S. et al. also found that the amount of chlorogenic acid was high in *Tragopogon porrifolius L.* and *Tragopogon tommasini* extracts, similar to our study [23, 24]. Moreover, the *Tragopogon dubius* methanolic extract exhibited a promising antioxidant effect [22]. Similarly we predict that it may show a promising antioxidant effect with values for FRAP at root and aerial parts extract in our study. In addition, we determined 157.8 µg of o-coumaric acid for aerial parts in our study. As Abdalla and Zidorn stated in their review on the use of *Tragopogon* species, their phytochemical and pharmacological properties [25], similar to our study, Smolarz and Krzaczek's studies found high apigenin, luteolin, syringic acid and caffeic acid levels in methanolic extracts of *Tragopogon orientalis L.* [26]. In this study, when evaluated in terms of DPPH radical scavenging activity, more activity was found in the aerial parts than in the root (0.60±0.01 mg/ml) (Fıgure 1). Similarly to our study, Farzei M. H. et al. found high DPPH activity and high phenolic content in the aerial parts of *Tragopogon graminifolius*. However, contrary to our study, they found high reducing power in the root and low reducing power in the above-ground parts in the FRAP analysis. (Table 1) [20]. Falahi E. et. al. investigated the phenol and flavonoid content and DPPH IC_{50} of leaves *Tragopogon graminifolium*. Based on their findings, the phenolic contents of extract of *T. graminifolium* were 513.71±60.77 mg GAE/ g dry extract and 7133.66±5368.17 µg/mL. [27]. Besides antioxidant capacity phytochemical composition of plants showed antimicrobial and antiquorum sensing activity. The problem of antibiotic resistance, which complicates the treatment of infectious diseases, has accelerated the studies on the use of plants and antimicrobial effective substances obtained from them. In addition, the inhibition of the bacterial communication system, which is effective in the management of virulence by many microorganisms, has been another focus in this struggle in recent years [28, 29]. Many synthetic and natural compounds have been studied and studied for the inhibition of the system that plays a role in the synthesis of many virulence factors such as elastase B production, biofilm formation, protease production, and swarming motility [30, 31]. In the literature review, no study was found in which the inhibition effect of on antiquorum sensing, especially the swarming motility of *Pseudomonas* and pyocyanin pigment production. However, in a study with antibacterial properties of *T. oligolepis* were investigated and it was observed that the ethyl alcohol extracts was most effective on some MDR Staphylococcus species [27].

In conclusion, in this study, phenolic content of *T. oligolepis*, which is an endemic species, were investigated and its antioxidant ability and some virulence factors in *P. aeruginosa*, were investigated. It was found that the bacterial test results showed an inhibition effect against pyocyanin production and swarming motility, which have an important role in virulence. In recent years, when antibiotic resistance is a serious problem, the tendency to herbal drugs in the fight against bacteria and the prevention of the formation of infectious diseases by preventing the communication between bacteria without killing them are seen among the promising strategies.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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