International Food Research Journal 27(1): 160 - 170 (February 2020)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidant, antimicrobial, and DNA protection activities of some Tanacetum species and phenolic richness in their ethanolic extracts

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Article history

Received: 3 September 2019
Received in revised form: 5 January 2020
Accepted: 10 January 2020

Keywords

Tanacetum, antioxidant, antimicrobial, DNA protection, phenolic

Abstract

Tanacetum species have been used traditionally for a long time due to their various biological activities such as anticoagulant, antimicrobial, cytotoxic, insecticidal, and antimigraine. In the present work, ethanolic extracts were prepared from aerial parts of four *Tanacetum* species (*T. parthenifolium, T. chiliophyllum, T. balsamitoides,* and *T. zahlbruckneri*) by Soxhlet Extraction Method; and 17 different phenolic concentrations of the extracts were determined by HPLC. Subsequently, antimicrobial effects were investigated with hollow agar method and *in vitro* antioxidant activities including total antioxidant activity, DPPH (2,2-diphenyl-1-picryl-hydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), radical scavenging activity, ferric iron (Fe^{3+}), and cupric (Cu^{2+}) reduction. Finally, protective effects on pBR322 plasmid DNA damaged by H_2O_2 using gel electrophoresis were investigated. Although a few phenolics were well known as antioxidant, it could not be detected in some of the extracts. The plant extracts however were generally found to have high phenolic content. In the antimicrobial studies, extracts of *T. parthenifolium* and *T. zahlbruckneri* did not have a lethal effect on *S. cerevisiae*. The extracts were also found to have powerful antioxidant properties. They exhibited similar activity with the standards, which are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol (α-Toc). In addition, they did not show useful effect on the damages of H_2O_2 and/or DMSO depending on the concentration. However, when extracts were applied alone, they partly stabilised the plasmid DNA. It was also observed that the high phenolic content of *T. chiliophyllum* extract had a positive effect on its antioxidant activity and DNA protective activity.

Introduction

Growing awareness about the side effects of synthetic drugs has led to the use of more natural antioxidant in the society (Branen 1975; Kusumawati *et al.,* 2018). According to the World Health Organization (WHO), approximately 70 - 95% of developing countries primarily benefit from plants for their primary health care (Talibi *et al.,* 2012). As a result of adverse environmental pollution such as cigarette smoke and uncontrolled drug use, increased concentration of cellular reactive oxygen species (ROS) such as H_2O_2 can damage the complex molecules such as DNA, and cause some serious diseases such as cancers (Vichi *et al.,* 2001; Al-Gubory, 2014; Budak *et al.,* 2014). Plant defence systems can also voluntarily produce ROS to cope with some pests and harmful microorganisms (Dinakar *et al.,* 2010). In numerous studies conducted on various plants, due to their secondary metabolites, plant extracts were reported to have antimicrobial and antioxidant activities (Sibanda and Okoh 2007; Sicari *et al.,* 2018) and secondary metabolites may also have a significant impact on the treatment of many diseases in the broad spectrum (Sicari *et al.,* 2018).

The Asteraceae family, which has the highest number of species in the world, is a large flowering plant family. This family represents 1,620 genera and more than 23,600 species (Ozturk and Cetin, 2013), of which 140 genera and 1,209 species are found in Turkey. A total of 447 of these species are endemic, and the endemism rate is 37% (Davis, 1988; Özhatay and Kültür 2006; Doğan 2007). Different species of *Tanacetum* genus of this family are known to be food preservatives. These species have been reported to be used in antiseptic, spasmolytic, antidandruff, and migraine treatment.

Plants have been discussed in the literature

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due to their medical and biological importance for many years. Although plants are currently used in alternative treatments and have partially prevented new discoveries, the discovered side effects of chemical drugs have accelerated the search for alternative treatments. Information about the biological properties of the plants used in the present work is quite limited. Therefore, the present work aimed to establish a foundation for advanced pharmacological and medical researches. For this purpose, four different *Tanacetum* species were collected at an elevation of 1650 - 1800 m from northern slope of Kambos mountain (38° 19' 41.93" N, 41° 59' 38.97" E); and the concentrations of 17 phenolics in ethanol extracts were determined using HPLC. In addition to this, the antimicrobial and *in vitro* antioxidant activities were investigated by various biological studies and thus, the plant literature was developed, and important data were obtained for new pharmacological studies.

Materials and methods

Plant collection and extract preparation

T. balsamitoides, T. parthenifolium, T. zahlbruckneri, and *T. chiliophyllum* were collected during the vegetation period between 2014-2015 from Bitlis province in Turkey (Figure 1). Herbarium material was formed from plants, and was stored in the Laboratory of Science and Technological Application and Research Centre, Bitlis Eren University. The ethanolic (EtOH) extracts were prepared using 50 g of the aerial parts of the plants, and were stored at -18°C in amber bottles.

Phenolic substance analysis by HPLC

Chromatograms were generated using HPLC to determine the number of the standards; and 17 different phenolic concentrations were calculated using the chromatograms and calibration curves that were prepared with intermediate stock solutions of standards. For this purpose, the extracts and standards were prepared at different concentrations (100, 75, 50, 25, and 10 mM) using the method of Seal (2016), and were filtered using the 0.45 µm membrane filter.

Microorganisms and sample preparation for antimicrobial study

A total of ten microorganisms which included bacteria *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus ATCC 25923*, *Bacillus megaterium* DSM 32, *Enterobacter aerogenes* ATCC 13048, *Eshericha coli* ATCC 11229, *Pseudomonas aeroginosa* 9027, and *Klebsiella pneumonia* 13883; andyeasts *Yarrowia lipolytica*, *Candida albicans* ATCC 10231, and *Saccharomyces cerevisiae* were obtained from the Central Research and Application Centre of Muş Alparslan University; and prepared/-maintained following the method of Hindler (1992). The concentration of bacteria and yeasts was adjusted according to the 0.5 standard of McFarland.

Antimicrobial activity method

The effects of the extracts and antibiotics on microorganisms were determined using the Hollow Agar method. Antibiotics used as the standard were purchased from Oxoid (UK). The antibiotics used were erythromycin; E-15, ampicillin/sulbactam; SAM-20, rifampicin; RD-5, amikacin; AK-30, and fluconazole; FCA-25. A 100 mg/mL concentrations of extracts and antibiotics were prepared, and the antimicrobial effects of 75, 100 and 150 µL were investigated following the method of Sagdic *et al.* (2003).

In vitro antioxidant studies

Total antioxidant activity

The total antioxidant activities were measured using the Ferric Thiocyanate method of Mitsuda *et al.* (1966). Based on this method, the absorbance values of the extracts and standards were spectrophotometrically measured at 500 nm until the control absorbance reached the maximum level.

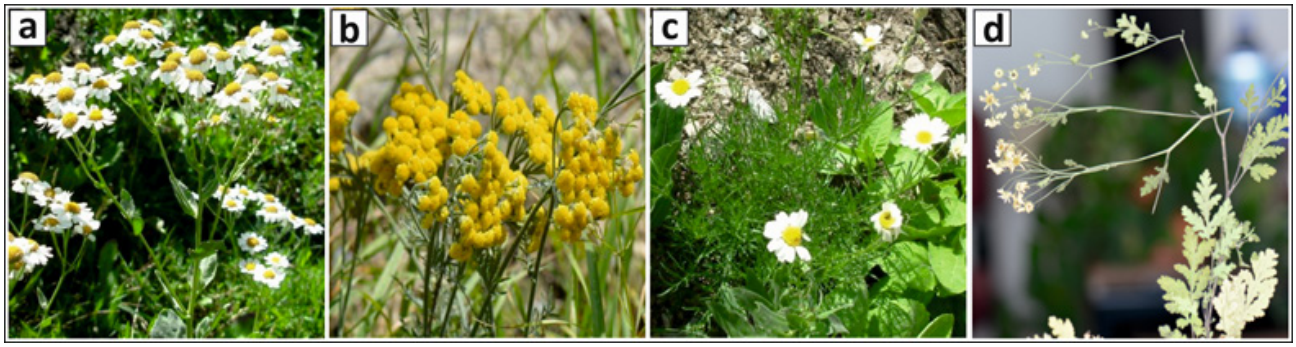

Figure 1. a) *Tanacetum balsamitoides*, b) *Tanacetum chiliophyllum* var. chiliophyllum, c) *Tanacetum zahlbruckneri*, and d) *Tanacetum parthenifolium.*

DPPH free radical scavenging activity

DPPH free radical scavenging activity was performed following the method of Blois (1958). Based on this method, the absorbance values of the extracts and standards were spectrophotometrically recorded at 517 nm at different concentrations (25, 50, and 100 µg/mL). DPPH radical scavenging percentages were then calculated using Equation 1:

$$\text{Scavenging Capacity of DPPH Radical (\%)} = \left(1 - \frac{\text{Sample absorbance}}{\text{Control absorbance}}\right) \times 10 \quad \text{(Eq. 1)}$$

ABTS radical scavenging activity

The method of Wu *et al.* (2009) was used to determine the ABTS radical scavenging activity. Based on this method, extracts and standards were prepared at different concentrations (25, 50, and 100 µg/mL); and their absorbance values were spectrophotometrically recorded at 505 nm with the phosphate buffer serving as blank.

Iron (Fe^{3+}) reduction activity

The total reduction power was determined following the method used by Alan *et al.* (2016). Forr this purpose, the extracts and standards were prepared at different concentrations (25, 50, and 100 µg/mL); and their absorbance values were spectrophotometrically recorded at 700 nm.

Cupric (Cu^{2+}) reduction activity

The Cu^{+2} reduction capacities of the standards and extracts were evaluated using the CUPRAC method modified by Gulcin (2006). A 0.25 mL of neocuprin solution, 0.01 M $CuCl_2$, and 1 M ammonium acetate buffer were transferred into test tubes, and then the different concentrations (25, 50, and 100 µg/mL) of standards and extracts were added on. The mixture was vortexed and incubated for 30 min. The reduction in cupric ions was spectrophotometrically recorded at 450 nm.

DNA protective activity of plant extracts

The DNA protective activity studies were performed following the method of Siddall *et al.* (2002). Protective activities of extracts were assayed on pBR322 plasmid DNA damaged by H_2O_2 at different concentrations (25, 50, and 100 mg/mL). For this purpose, 5 µL of prepared extract and 5 µL loading buffer were mixed together, and were loaded to electrophoresis with the other electrophoresis components (Table 1). The agarose gel electrophoresed at 40 V for 2 h was stained with ethidium bromide and visualised using Londershausen (1996) imaging system.

Statistical analysis

In vitro antioxidant powers and antimicrobial effects of plant extracts were investigated; and the data were statistically compared with the results of the standards. All tests were repeated three times, and the results were compared using One-way ANOVA followed by Dunnett's Multiple Comparisons Test, GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California, USA. All statistical data were represented as means ± Standard Errors of the Mean (SEM); and $p < 0.05$ was considered significant.

Table 1. The amounts and components of the samples for electrophoresis.

DNA (µL)	H_2O_2 (µL)	DMSO (µL)	Extract (10 µL) and concentration	PW (µL)	Total Volume (µL)
10	N/A	N/A	N/A	15	25
10	5	N/A	N/A	10	25
10	5	10	N/A	N/A	25
10	N/A	10	N/A	5	25
10	5	N/A	Leaf/Root EtOH (100 mg/mL)	N/A	25
10	5	N/A	Leaf/Root EtOH (50 mg/mL)	N/A	25
10	5	N/A	leaf/Root EtOH (25 mg/mL)	N/A	25
10	N/A	N/A	Leaf/Root EtOH (100 mg/mL)	5	25
10	N/A	N/A	Leaf/Root EtOH (50 mg/mL)	5	25
10	N/A	N/A	Leaf/Root EtOH (25 mg/mL)	5	25

N/A: No addition.

Table 2. The concentrations of phenolics as μg/mL in the ethanolic extracts obtained from the aerial parts of four *Tanacetum* species (*T. chiliophyllum*, *T. parthenifolium*, *T. balsamitoides*, *T. zahlbruckneri*).

Phenolic	*T. chiliophyllum*	*T. parthenifolium*	*T. balsamitoides*	*T. zahlbruckneri*
Ascorbic acid	41.475	35.295	0.759	2.796
Gallic acid	N/A	17.108	N/A	N/A
Myricetin	15.236	79.778	1.812	34.024
Abscisic acid	182.886	7.114	4.162	1.941
Quercetin	4.314	6.139	11.397	1.962
Apigenin	242.203	249.155	433.027	6.951
Kaempferol	170.790	6.779	1.084	1.949
Curcumin	5.500	74.954	16.453	4.649
Catechol	0.483	17.186	6.845	23.446
Vanillin	1.223	N/A	N/A	2.862
Caffeic acid	N/A	7.719	4.161	10.167
Cinnamic acid	0.886	6.612	4.712	1.584
Rosmarinic acid	3.340	1.493	8.986	7.774
Salicylic acid	N/A	N/A	0.057	N/A
3,4-Dihydroxybenzoic acid	N/A	N/A	N/A	N/A
4-Hydroxybenzoiic acid	5.331	2.414	2662.21	2.214
Trans-*p*-coumaric acid	3.217	28.079	2.455	8.463

N/A: Not Available.

Results and discussion

Phenolic content results by HPLC

Phenolic concentrations were determined using HPLC standards. Phenolic concentrations are shown in Table 2.

The amounts of catechol and apigenin were detected to be the lowest and highest at 0.483 and 242.203 μg/mL, respectively, in *T. chiliophyllum* extract. In addition to this, gallic acid, caffeic acid, salicylic acid, and 3,4-Dihydroxybenzoic acid could not be detected in the same extract.

In *T. parthenifolium* extract, contrary to the inability to detect vanillin, salicylic acid, and 3,4-dihydroxybenzoic acid, the amounts of apigenin and rosmarinic acid were detected to be the highest and the lowest at 249.155 and 1.493 μg/mL, respectively. Vanillin and 3,4-dihydroxybenzoic acid could not be detected, but the amount of apigenin was the highest (433.027 mg/mL), and the amount of salicylic acid was the lowest (0.057 mg/mL) in *T. balsamitoides* extract.

Gallic acid, salicylic acid, and 3,4-dihydroxybenzoic acid could not be detected; however, the highest and the lowest amounts of myricetin and cinnamic acid were detected as 34.024 and 1.584 mg/mL, respectively in *T. zahlbruckneri* extract.

In previous studies conducted on different species of *Tanacetum* genus, it was reported that gentisic acid, luteolin, caffeic acid, and chlorogenic acid were found at small amounts in HPLC-MS analysis results. In the same study, the extract was reported to have the highest amount of quercetin (27.61 ± 0.39 μg/g), and was followed by apigenin (9.71 ± 0.18 μg/g) (Hanganu *et al.,* 2016). In another study conducted on three different species of *Tanacetum* by LC-MC, in addition to the failure to detect kaempferol and apigenin in all extracts, quercetin was reported to be detected in only *T. vulgare* extract (Ivânescu *et al.,* 2018). Another study conducted on two different extracts obtained from *T. vulgare* reported the trace amounts of caffeic acid and kaempferol, and the presence of quercetin in both extracts (Mureşan *et al.,* 2015).

In the present work, salicylic acid was detected in all extracts, and apigenin could not be detected in *T. zahlbruckneri* extract; however, gallic acid could only be detected in *T. parthenifolium* extract. Overall, phenolic content of all extracts was very high, but the phenolic content of *T. chiliophyllum* extract was higher than in the other extracts.

Antimicrobial results of plant extracts

Positive results were obtained in studies on antimicrobial effects; and striking data were obtained in the statistical evaluation of inhibition diameters. The effect of 150 mL ethanolic extract on the microorganisms was better than the other amounts of extracts (75 and 100 μL) and the antibiotics. The results of 75 and 100 μL are not shown; and 150 μL extract results and statistical comparisons are shown in Table 3.

Table 3. Effects of 150 µL extracts, antibiotics, and statistical evaluation.

Organism		Antibiotics		Erythromycin	Ampicillin/ Sulbactam	Amikacin	Rifampicin	Fluconazole
Gram-Positive	*B. subtilis*	**Antibiotic results**		**20 ± 0.00**	**14 ± 1.15**	**11 ± 1.00**	**21 ± 0.00**	-
		T. chiliophyllum	-	-	-	-	-	N/A
		T. parthenifolium	16 ± 0.57	-****	+*	+****	-****	N/A
		T. balsamitoides	13 ± 0.00	-****	ns	+*	-****	N/A
		T. Zahlbruckneri	14 ± 0.57	-****	ns	+***	-****	N/A
	S. aureus	**Antibiotic results**		**21 ± 1.00**	**10 ± 0.00**	**9 ± 0.00**	**18 ± 1.15**	-
		T. chiliophyllum	15 ± 1.00	-****	+****	+***	-****	N/A
		T. parthenifolium	23 ± 1.73	ns	+****	+****	ns	N/A
		T. balsamitoides	12 ± 0.00	-****	ns	ns	-****	N/A
		T. Zahlbruckneri	14 ± 0.00	-****	+***	+**	-****	N/A
	B. megaterium	**Antibiotic results**		**25 ± 0.00**		**10 ± 1.00**	**16 ± 0.00**	
		T. chiliophyllum	14 ± 0.00	-****	N/A	+*	****	N/A
		T. parthenifolium	18 ± 1.00	-****	N/A	+****	+*	N/A
		T. balsamitoides	14 ± 0.00	-****	N/A	+****	-*	N/A
		T. Zahlbruckneri	19 ± 1.00	-****	N/A	+****	+***	N/A
	E. aerogenes	**Antibiotic results**		**27 ± 1.00**	**10 ± 1.00**	**9 ± 0.00**	**16 ± 1.00**	**-**
		T. chiliophyllum	18 ± 0.57	-****	+****	+****	ns	N/A
		T. parthenifolium	17 ± 1.15	-****	+****	+****	ns	N/A
		T. balsamitoides	12 ± 0.57	-****	ns	+**	-****	N/A
		T. Zahlbruckneri	21 ± 1.15	-****	+****	+****	+****	N/A
Gram-Negative	*E. coli*	**Antibiotic results**		**19 ± 1.52**	**13 ± 0.00**	**13 ± 0.00**	**18 ± 0.00**	**-**
		T. chiliophyllum	18 ± 1.52	ns	+****	+****	ns	N/A
		T. parthenifolium	19 ± 0.57	ns	****	****	ns	N/A
		T. balsamitoides	16 ± 1.00	-**	+**	+**	-*	N/A
		T. Zahlbruckneri	12 ± 0.00	-****	ns	ns	-****	N/A
	P. aeroginosa	**Antibiotic results**		**19 ± 0.00**		**14 ± 1.15**	**8 ± 0.00**	-
		T. chiliophyllum	14 ± 0.00	-****	N/A	ns	+****	N/A
		T. parthenifolium	18 ± 1.52	ns	N/A	+***	+****	N/A
		T. balsamitoides	18 ± 0.00	ns	N/A	+***	+****	N/A
		T. Zahlbruckneri	17 ± 1.52	ns	N/A	+**	+***	N/A
	K. pneumonia	**Antibiotic results**		**19 ± 1.73**	**16 ± 0.57**	**10 ± 0.00**	**19 ± 1.73**	-
		T. chiliophyllum	15 ± 1.15	-**	ns	+***	-**	N/A
		T. parthenifolium	15 ± 1.73	-****	-*	+*	-****	N/A
		T. balsamitoides	13 ± 0.00	-****	-**	ns	-****	N/A
		T. Zahlbruckneri	12 ± 0.00	ns	ns	+**	ns	N/A

		Antibiotic results		-	-	-	-	**21 ± 0.00**
Fungus	*Y. lipolytica*	*T. chiliophyllum*	26 ± 1.00	N/A	N/A	N/A	N/A	+***
		T. parthenifolium	23 ± 1.00	N/A	N/A	N/A	N/A	ns
		T. balsamitoides	17 ± 1.00	N/A	N/A	N/A	N/A	-***
		T. Zahlbruckneri	22 ± 1.00	N/A	N/A	N/A	N/A	ns
		Antibiotic results		-	-	-	-	**23 ± 1.52**
	C. albicans	*T. chiliophyllum*	23 ± 1.73	N/A	N/A	N/A	N/A	ns
		T. parthenifolium	24 ± 0.57	N/A	N/A	N/A	N/A	ns
		T. balsamitoides	21 ± 1.73	N/A	N/A	N/A	N/A	ns
		T. Zahlbruckneri	25 ± 0.57	N/A	N/A	N/A	N/A	ns
		Antibiotic results		-	-	-	-	-
	S. cerevisiae	*T. chiliophyllum*	23 ± 0.57	N/A	N/A	N/A	N/A	N/A
		T. parthenifolium	-	N/A	N/A	N/A	N/A	N/A
		T. balsamitoides	23 ± 1.00	N/A	N/A	N/A	N/A	N/A
		T. Zahlbruckneri	-	N/A	N/A	N/A	N/A	N/A

Note: N/A: Not Available; ─: No inhibition zone; +: Higher extract activity than antibiotics; -: Higher antibiotic activity than extract. * is the significance symbol in the comparison of the effects of antibiotics and extracts; *: $p < 0.05$ (significant); **: $p < 0.01$ (very significant); ***: $p < 0.001$; ****: $p < 0.0001$ (extremely significant); ns: $p > 0.05$ (not significant). Data are means ± SEM of three measurements ($n = 3$); All p values were derived from Dunnett's Multiple Comparisons Test, One Way ANOVA.

In addition to the fact that the antimicrobial activity of *T. chiliophyllum* extract was not observed on *B. subtilis*, it showed the highest activity on *Y. lipolytica* (26 ± 1.00), and the lowest activity on *P. aeroginosa* and *B. megaterium* (14 ± 0.00; for both of them).

When the results of *T. parthenifolium* extract were examined, it was seen clearly that there was no activity on *S. cerevisiae*; and it was also found that the extract had the highest activity on *C. albicans* (24 ± 0.57 mm), and the lowest activity on *K. pneumonia* (15 ± 1.73).

It was detected that the extract had the highest activity on *S. cerevisiae* and the lowest activity on *S. aureus* and *E. aerogenes* (23 ± 1.00 and 12 ± 0.00, respectively) in the results of *T. balsamitoides* extract.

In addition to the ineffectiveness of *T. zahlbruckneri* extract on *S. cerevisiae*, in contrast to its highest activity on *C. albicans* (25 ± 0.57 mM), the lowest activity was determined on *E. coli* and *K. pneumonia* (12 ± 0.00; for both of them). In addition to these, a significant increase was observed in the antimicrobial activity depending on extract concentration.

T. corymbosum extract was found to have the best antimicrobial activity on *S. aureus*, but did not show any activity on *E. coli* and *P. aeruginosa*. In addition, parallel antifungal activities of extracts on *C. albicans* and *C. parapsilosis* were among the data that were reported in the same study (Ivânescu *et al.,* 2018).

In a study conducted on different extracts of *T. kotschyi*, in addition to high antimicrobial effect of the plant extract prepared from the aerial parts on *C. parapsilosis*, the poor activity of other extracts on all microorganisms was emphasised (Öztekin, 2010). Meanwhile, *T. vulgare* extract was reported to have poor antibacterial activity on *B. subtilis*, and did not have any activity on the *C. albicans*, *E. coli*, and *P. aeruginosa* (Mureşan *et al.,* 2015).

In the present work, the activity of *T. chiliophyllum* extract on *B. subtilis* and the activities of *T. parthenifolium* and *T. zahlbruckneri* extracts on *S. cerevisiae* could not be detected. The increase in the antimicrobial activity was shown to be related to extract concentration. In a previous study conducted on the *T. zahlbruckneri*, hexane extract was reported to have no activity on *E. coli* and *P. vulgaris*, and on *A. niger*. In the same study, it was stated that the extracts were compared in terms of the effects of chloramphenicol; and they showed moderate activity on some Gram-positive bacteria. It was also emphasised that plant extracts exhibited similar activity with ketoconazole (Çaglar, 2011).

When the effects of extracts and fluconazole used in the present work were compared with each other, in addition to similar antifungal activity, the extracts were found to have partly similar activity with erythromycin and rifampicin, and showed better

antibacterial activity than ampicillin and amikacin. In this respect, although the phenolic and total antioxidant activity of *T. chiliophyllum* extract was higher than the other plant extracts, significant increase was observed in its antimicrobial effect. In addition to this, the antifungal effect of all plant extracts was more pronounced than their antibacterial effect.

In vitro antioxidant study results

Total antioxidant activity results

Total antioxidant activity was investigated at different concentrations (25, 50, and 100 µg/mL), and since the most effective activity was observed at 100 µg/mL concentration, statistical comparison was only performed for 100 µg/mL concentrations (Figure 2a). The percentages of the standard and extracts in the inhibition of linoleic acid emulsion were calculated using Equation 2 when control absorbance value reached the maximum level.

$$\text{Lipid Peroxidation Inhibition (\%)} = 100 - \left(\frac{A_{sample}}{A_{control}} \times 100\right) \quad \text{(Eq. 1)}$$

where, A_{sample} = value of the sample absorbance at the incubation time at which control absorbance is maximum; and $A_{control}$ = value of control absorbance at the same incubation time.

Peroxidation percentages of linoleic acid emulsion for 100 µg/mL were as follows: *T. chiliophyllum* (78.72%), *T. parthenifolium* (65.96%), *T. balsamitoides* (68.51%), and *T. zahlbruckneri* (69.78%), respectively; and the percentages of standard antioxidants were as follows: butylated hydroxyanisole (BHA) (72.34%), butylated hydroxytoluene (BHT) (72.76%), and α-tocopherol (α-Toc) (57.87%), respectively.

Methanol-dichloromethane extract was reported to have power total antioxidant capacity in a study on different extracts of *T. abrotanifolium*

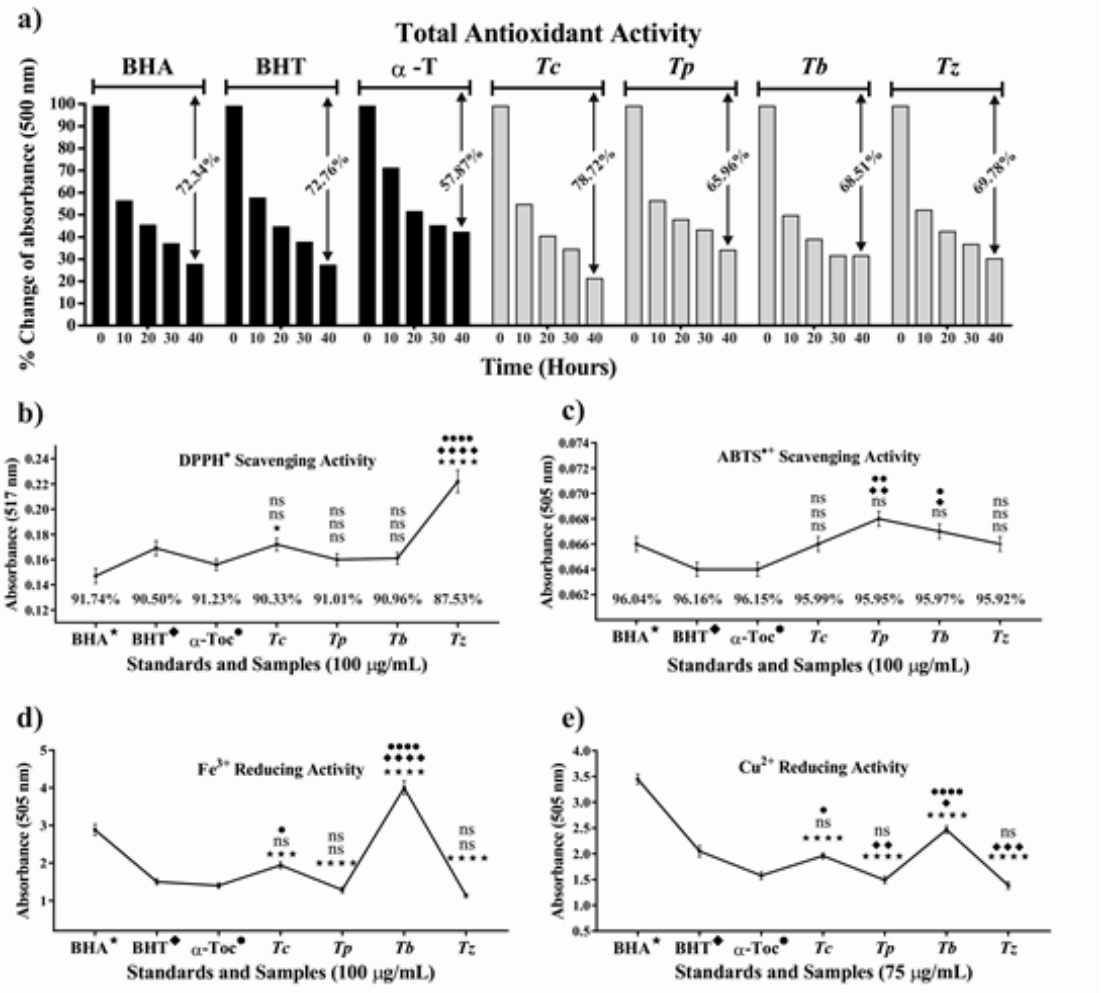

Figure 2. a) % Change in total antioxidant activity absorbance by thiocyanate method, b) DPPH radical scavenging results, c) ABTS radical scavenging results, d) Fe^{3+} reducing power results by FRAP, and e) Cu^{2+} reducing power results by CUPRAC. Standard antioxidants: BHA, BHT, and α-Toc; Extracts: Tc, Tp, Tb, and Tz. In the comparison of standards and extracts, " " for BHA, " " for BHT, and "●" for α-Toc are used as a symbol of statistical significance. $p < 0.05$: a symbol (significant); $p < 0.01$: two symbols (very significant); $p < 0.001$: three symbols; and $p < 0.0001$: four symbols (extremely significant); and $p > 0.05$: "ns" for all non-significant comparisons (not significant). Significance symbols are also shown above of standards. Data are means ± SEM of three measurements ($n = 3$). All p values are derived from Dunnet's multiple comparisons test, One Way ANOVA.

(Gecibesler, 2017). In the present work, all extracts exhibited close or better activity than the standards. The results of the present work are parallel with the aforementioned studies. In addition, phenolic content of *T. chiliophyllum* extract was higher than *T. parthenifolium* extract. A similar increase was observed in terms of the total antioxidant activity and total reduction power. These data showed that the phenolic content positively reflected the antioxidant activity; and the plant extracts can be said to be powerful antioxidant sources.

Scavenging results of DPPH free radical

DPPH radical scavenging activity was repeated for different concentrations; and close activities to the standard antioxidants were detected at 100 µg/mL concentration. A 100 µg/mL concentration of standard antioxidants and extracts were compared statistically (Figure 2b). DPPH radical scavenging percentages for 100 µg/mL concentrations were as follows: BHA (91.74%) ≥ α-Toc (91.23%) ≥ *T. parthenifolium* (91.01%) ≥ *T. balsamitoides* L. (90.96%) ≥ BHT (90.50%) ≥ *T. chiliophyllum* (90.33%) > *T. zahlbruckneri* (87.53%).

A study on *T. balsamita* emphasised that the DPPH radical scavenging activity was quite high and the reason for this was attributed to the phenolic content of the plant (Bączek *et al.,* 2017). Another study emphasiaed that the high percentage of essential oils in *T. parthenium* showed DPPH radical scavenging activity and the usability of this plant as natural antioxidant (Rezaei *et al.,* 2017). A study on *T. kotschyi* whose results are similar to this study reported that methanol extracts have similar activity to the standards (Oztekin, 2010). Based on the results of the present work, the effect of *T. balsamitoides* extract can be said to be quite high due to its phenolic content.

Scavenging results of ABTS radical

ABTS radical scavenging activities were detected to be both very high and close to each other; and the 100 µg/mL results of the extracts and standards were statistically compared (Figure 2c). ABTS radical scavenging percentages for the 100 µg/mL were as follows: BHT (96.16%) ≥ α-Toc (96.15%) ≥ BHA (96.04%) ≥ *T. chiliophyllum* (95.99%) ≥ *T. balsamitoides* L. (95.97%) ≥ *T. parthenifolium* (95.95%) ≥ *T. zahlbruckneri* (95.92%).

A study on essential oils of *T. macrophyllum* expressed that ABTS radical scavenging activity of the essential oils was higher than Trolox antioxidant activity (Venditti *et al.,* 2018). By considering the similar activities in the results of the standard and extracts, it can be concluded that the results obtained in the present work parallel that of the aforementioned literature.

Reduction results of iron (Fe^{3+}) cations

Based on the results obtained in the present work, total reduction power was usually observed to increase depending on the concentration which showed more significant effect at 100 µg/mL concentration in the order of *T. balsamitoides* > BHA > *T. chiliophyllum* > BHT > α-Toc > *T. parthenifolium* > *T. zahlbruckneri* (Figure 2d).

In a study on the biological activities of different extracts of *T. basalum* species, it was reported that except for the dichloromethane extract, the reduction capacities of the other extracts were better than the results of the standards (Erdogan, 2012). A study on *T. macrophyllum* reported that ethanol extract had a very strong reducing capacity (Venditti *et al.,* 2018). In the present work, *T. balsamitoides* extract was found to exhibit better activity than the standards and the other extracts, and this agrees with those of the other studies previously mentioned.

Reduction results of Cupric (Cu^{2+}) Cations

Cupric reduction capacities were performed by measuring the absorbance values of extracts that had different concentrations (25, 50, and 75 µg/mL); and the increase in cupric reduction capacities depending on concentration were observed. In addition to this, the most effective reduction was observed at 75 µg/mL concentration. The results were compared statistically with the standards (Figure 2e). It is apparent that *T. balsamitoides* and *T. chiliophyllum* extracts exhibited similar activities to those of BHA and BHT in the most concentrations. On the contrary, the results of *T. parthenifolium* and *T. zahlbruckneri* were quite low as compared to the standards. Briefly, *T. chiliophyllum* and *T. balsamitoides* ethanolic extracts were found to be good cupric reductive agents; and because of this, these plants can be said to be good antioxidants.

DNA protection activity results

From the simplest molecules like H_2O_2 to the most complex materials such as polymers, various molecules can act as radicals and affect DNA, proteins and lipids. These interactions constitute the starting point for many neurodegenerative diseases such as cancers, cardiovascular diseases, and cataract (Sicari *et al.,* 2018). The double stranded helix structure of the DNA is known as Form I, which can be transformed into Form II by breaking

the single chain of DNA with the interaction of these molecules with DNA. Form I moves faster than Form II on agarose gel electrophoresis. The other chain of the DNA may also break as a result of more advanced interaction, and Form III may occur. Form III is known as the linear structure of the DNA, and its movement on agarose gel is faster than Form II. These three structures as stained by ethidium bromide can be visualised under UV light (Zhang *et al.,* 2001).

In the gel image of *T. chiliophyllum* and *T. parthenifolium* obtained in the present work, in addition to the degradation of Form I by H_2O_2, when H_2O_2 and DMSO were added together, complete destruction was observed in all three structures. In addition, it can be said that DMSO alone made Form I and Form II more stable; and *T. chiliophyllum* and *T. parthenifolium* did not show significant effects on the DNA depending on the concentration (Figure 3). However, it can be said that *T. chiliophyllum* extract was effective in the stabilisation of Form II in all concentrations. The reason for this effect may be due to its high phenolic content and total antioxidant activity. It is also noteworthy that in the samples in which extracts alone were added, Form I was transformed into Form II (Figure 3, line 8, 9, 10, 14, 15, and 16). Although not known exactly, the reason for this may be due to the change of a plant component or its activation during the study or intensive radical production by a protective mechanism. *T. zahlbruckneri* extract alone can be said to be effective in the stabilisation of Form II; however, it is clear that *T. balsamitoides* extract had no positive effect on the degraded DNA structure (Figure 3, line 24, 25, 26, 30, 31, and 32).

A study on *Cyanthillium cinereum*, which is also a member of the Asteraceae family, reported that DNA was protected by plant water extracts against the damage caused by H_2O_2 (Guha *et al.,* 2011). Another study reported that the extracts obtained from aerial parts of *I. oculus-christi* played a protective role on the pBR322 plasmid DNA damaged by H_2O_2 and UV (Berk *et al.,* 2011).

When four different plant extracts, H_2O_2 and DMSO were applied together on plasmid DNA, the harmful effect of H_2O_2 was not eliminated. Even when the extracts were applied alone, Form I was transformed into Form II. In addition to this, it was clear that especially *T. chiliophyllum* and *T. zahlbruckneri* extracts had a positive effect on stabilisation of Form II structure. Although the DNA protection properties of the extracts were weak, their positive effects on microorganisms support the radical production idea. That is, the current intense concentration of radicals may be responsible for the death of microorganisms.

Conclusion

Although a few of the well-known phenolic antioxidants such as salicylic acid, caffeic acid, and ascorbic acid could not be detected in some extracts, the diversity and high amounts of other phenolics in the extracts are important evidence that they are

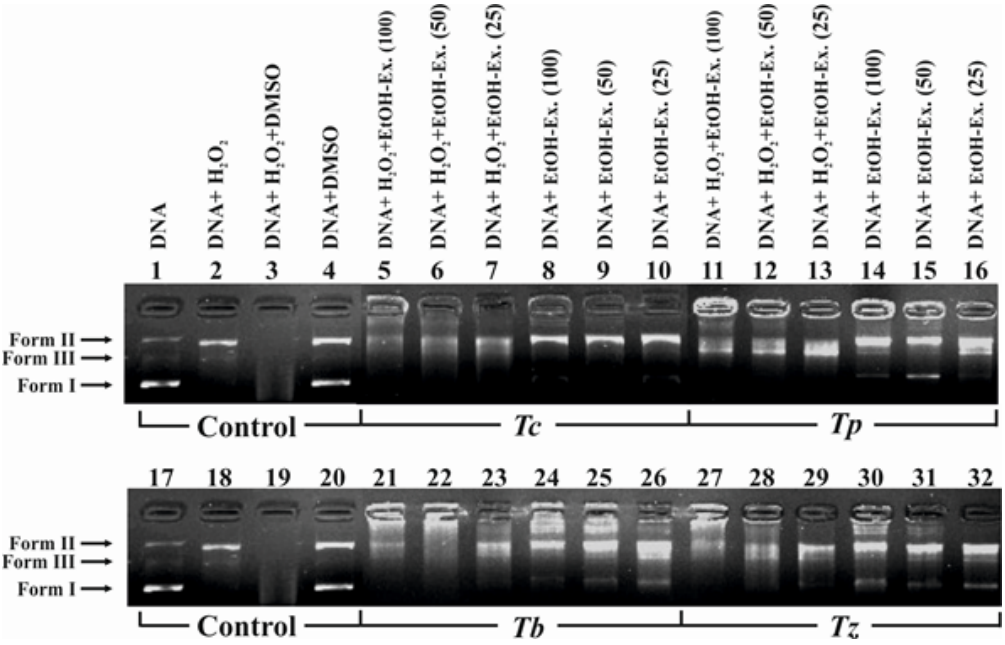

Figure 3. Agarose gel electrophoresis image showing the effects of four different plant extracts on pBR322 plasmid DNA, Ta: *Tanacetum chiliophyllum*, Tp: *Tanacetum parthenifolium*, Tb: *Tanacetum balsamitoides*, Tz: *Tanacetum zahlbruckneri*, and EtOH-Ex: Ethanol extract.

natural antioxidants. It was also detected that *T. chiliophyllum* and *T. parthenifolium* extracts had good antimicrobial activities; and were also detected to have no lethal activity on *S. cerevisiae*. *T. chiliophyllum* extract showed strong antioxidant activity *in vitro*, and its phenolic content was shown to have a positive effect on the antioxidant activity and DNA protective activity. Additionally, the extracts had no effect on the scavenging of the harmful effects of DMSO and H_2O_2, but when applied alone, majority were found to have a positive effect on the stabilisation of Form II. The present work has great importance for future studies due to the positive effects determined on microorganisms, high *in vitro* antioxidant activities, and phenolic richness.

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