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Investigation of Genotoxic, Antimicrobial and Antioxidant Activities of Leaf and Flower Extracts of *Cynara syriaca* Boiss

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ABSTRACT

The mutagenicity and antimutagenicity of leaves and flower extract of *Cynara syriaca* Boiss were studied with Ames assay in *Salmonella typhimurium* TA98 and TA100 strains. While leaves extract did not show any mutagenic effects against all the tester strains with or without metabolic activation, the flower extract showed mutagenic effect against TA98 strain without metabolic activation. On the other hand, it has been observed that the extracts have antimutagenic activity against mutations induced by sodium azide and daunomycin. The antimicrobial activity of extracts was determined by disc diffusion and MIC value. Both of the extracts possess weak antimicrobial activity. Cupric reducing antioxidant capacity (CUPRAC), DPPH free radical scavenging activity, and ABTS radical cation decolorization methods were carried out to determine the antioxidant activity. Among the tested antioxidant methods, the highest antioxidant capacity was determined in ABTS radical cation decolorization assay in which both of the extracts exhibited the best effect. Flower extract exhibited higher activity also in DPPH free radical scavenging.

Keywords: Mutagenicity; Antimutagenicity; Antioxidant activity; Antimicrobial activity; *Cynara syriaca*

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1. Introduction

Plants have significant biological content and in recent years plant polyphenols are increasingly attracted to the role of antioxidants, mutagenic, antimutagenic and anticancerogenic properties as well as the prevention of several diseases such as cancer and cardiovascular diseases (Chulasiri 1998; Caderni et al 2000; Lin et al 2009; Sun et al 2011).

One of the secondary metabolites commonly found in fruits and vegetables is flavonoids. These polyphenol compounds are responsible for the color of many vegetables and fruits and at the same time provide important functions in reproduction and breeding plants. They act as defensive mechanisms against pathogens, parasites, and ruptures. (Báidez et al 2007). Natural phenolic compounds which were included flavonoids, phenolic acids, stilbenes, curcuminoids, tannins, lignans, quinones, coumarins and others have been reported to possess potent antioxidant activity and anticarcinogenic, antimutagenic, antiatherosclerotic, antibacterial,

antiviral anti-inflammatory activities (Owen et al 2000; Veeriah et al 2006; Báidez et al 2007; Han et al 2007). Phenolic compounds have contributed to the induction of apoptosis by arresting the cell cycle. Moreover, they inhibit DNA binding, regulating carcinogen metabolism, cell adhesion, migration, proliferation or differentiation and blocking signaling pathways (Huang et al 2009).

Researches on traditionally used plants have been increasing day by day and very important results have obtained from them. *Cynara syriaca* has been grown wild in a very narrow area in Southern Turkey (Kupicha 1975). There has been no chemical investigation on *C. syriaca* except for the lipid content of the seeds (Heidari et al 2000).

The aims of this study were to investigate the mutagenic, antimutagenic, antioxidant and antimicrobial activity of leaf and flower extracts of *C. syriaca*.

2. Materials and Methods

2.1. Plant extraction

The leaf and flower of *C. syriaca* was picked up from Diyarbakır, Turkey in June 2010 and identified by Prof. Dr. A. Selçuk ERTEKIN (Department of Biology, Faculty Science, Dicle University). The dried and powdered leaf and flowers of *C. syriaca* macerated with methanol at room temperature for 24 h. The solvent was evaporated after filtration and kept at +4 °C in a glass bottle.

2.2. Determination of minimum inhibitory concentration (MIC) and antimicrobial activity

Gram-positive bacteria (*Streptococcus pyogenes* ATCC19615 and *Staphylococcus aureus* ATCC 25923), gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922) and yeast (*Candida albicans* ATCC10231) which were purchased from Refik Saydam Sanitation Center (Turkey) were used for detecting the antimicrobial activity of the samples. The disc diffusion method was employed for this purpose ampicillin and fluconazole were used as positive controls for bacteria and yeast, respectively. The minimum inhibitory concentration of the extracts was determined by broth dilution methods (NCCLS 2009).

2.3. Determination of total phenolic and flavonoid contents

The total phenolic (Slinkard & Singleton 1977) and flavonoid contents (Moreno et al 2000) of extracts were expressed as gallic acid and quercetin equivalents, respectively, and calculated according to the following equations.

Absorbance= 0.1741 Gallic acid (µg) - 0.0224 (R^2= 0.9925)

Absorbance= 0.2784 Quercetin (µg) - 0.2872 (R^2= 0.9911)

2.4. Antioxidant activity assays

DPPH free radical scavenging activity (Blois 1958), cupric reducing antioxidant capacity (CUPRAC) (Apak et al 2004) and ABTS radical cation decolorization (Re et al 1999) methods were carried out to determine the antioxidant activity. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as positive controls. The percentage of inhibition was calculated by using the following equation except in the CUPRAC method in which increasing absorbance refers to increasing activity.

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

2.5. Mutagenic and antimutagenic activity

The bacterial mutagenicity and antimutagenicity assays were carried out according to Maron & Ames (1983). The plate incorporation procedure was done to detect reverse mutations from histidine dependence to histidine independence via *S. typhimurium* test strains TA98 and TA100 absence and presence S9 mammalian liver homogenate fraction. The NaN_3 (into distilled water–1.5 µg mL^{-1}) for *S. typhimurium* TA100, 2-aminofluorene requiring metabolic activation (in DMSO-0.1 mg mL^{-1}) and daunomycin (in distilled water- 6 µg mL^{-1}) for *S. typhimurium* TA98 were used as positive controls and 10% DMSO as the negative control.

While the mutagenicity assessment was conducted by a dose-response and a two-fold increase in the number of revertants, the antimutagenicity was assessed by the inhibition percentage of mutagenicity (IP) calculated by the following Equation:

$$IP(\%) = (1 - (A-B) / (C-B)) \times 100$$

Where; A, number of revertants on test plates incubated with mutagen and extract; B, spontaneous revertants (test strains incubated in the absence of both extract and mutagen); C, number of revertants on control plates incubated with the mutagen alone.

Forty percent or more inhibition was considered as strong antimutagenicity; twenty-five to forty percent inhibition was defined as moderate antimutagenicity and twenty-five or less inhibition as no antimutagenicity (Ikken et al 1999).

2.6. Statistical analysis

The results of the antimicrobial and antioxidant activity assays are expressed as mean ± SD of three experiments. The statistical significance was estimated using analysis of variance (ANOVA), Followed by a Dunnett's test to compare the treated groups to the control group. p values less than or equal to 0.05 were considered to indicate statistically significance.

3. Results and Discussion

3.1. Antimicrobial activity

The antimicrobial activities of *C. syriaca* leaf and flower extracts against different microorganisms were assessed according to the inhibition zone diameter. Results are given in Table 1. According to the results, both of the extracts possess weak antimicrobial activity (inhibition zone < 12 mm). The highest activity was observed in the leaf extract against *C. albicans* with 12.5±0.7 mm inhibition zone diameter and 250 µg mL^{-1} MIC value.

3.2. Total phenolic and flavonoid contents

Total phenolic and flavonoid contents of the extracts were determined as gallic acid (GAEs) and quercetin (QEs) equivalents, respectively. As depicted in Table 2, the flavonoid contents of the extracts higher than their phenolic contents. The phenolic content of flower extract found to be higher than leaf extract.

3.3. Antioxidant activity

Free radicals play a significant role in a variety of pathological markers and involved in many syndromes such as aging, atherosclerosis, and diabetes in humans. Antioxidants neutralize the free radicals by donating electron and protect from several progressive diseases (Nimse & Pal 2015).

Table 1- Zones of growth inhibition (mm) and MIC values of leaf and flower extracts of *C. syriaca* compared to positive controls

Samples		*Microorganisms*				
		Gram positive		*Gram negative*		*Yeast*
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albicans*
Leaf	[a]DD	9.6±0.8	8.5±0.7	11.5±0.7	10.5±0.7	12.5±0.7
	MIC	600.0±0.1	800.0±0.4	250.0±0.2	500.0±0.2	250.0±0.5
Flower	[a]DD	8.5±0.7	8.6±0.2	9.5±0.1	9±0.0	10.0±0.0
	MIC	800.0±0.5	800.0±0.5	600.0±0.1	600.0±0.7	600.0±0.1
Positive controls	[b]DD	35.0±0.2	19.0±0.2	20.0±0.1	-	30±0.3
	MIC	1.95±0.3	7.8±0.1	7.8±0.4	-	3.1±0.2

-, not active; [a]DD, inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 30 mg mL^{-1} of plant extracts; [b]DD, inhibition zone in diameter (mm) of positive controls that are ampicillin for bacteria and fluconazole for yeast. Minimum inhibitory concentration (MIC) values are given as µg mL^{-1}

Table 2- Total phenolic-flavonoid contents and antioxidant activity[a] of the extracts and positive controls

Samples	*Phenolic content (μg GAEs mg extract^{-1})[c]*	*Flavonoid content (μg QEs mg extract^{-1})[d]*	*IC_{50} (μg mL^{-1})*	
			DPPH Free Radical	*ABTS Cation Radical*
Leaf	4.59±0.17	52.95±0.51	6.13±0.70	2.64±0.50
Flower	7.29±0.28	52.47±0.17	2.34±0.49	1.91±0.20
BHT[b]	-	-	58.86±0.50	13.25±0.27
BHA[b]	-	-	7.88±0.20	17.59±0.10

[a], values expressed are means ± SEM of three parallel measurements ($P<0.05$); [b], positive controls; [c], *GAEs*, gallic acid equivalents ($y= 0.1741 x + 0.0224$ $R^2= 0.9925$); [d], *QEs* quercetin equivalents ($y= 0.2784 x - 0.2872$, $R^2= 0.9911$)

The results of DPPH and ABTS assays are expressed as IC_{50} value in Table 2. Lower IC_{50} value indicates the higher activity. The flower extract exhibited excellent antioxidant activity in both DPPH and ABTS assays with 2.34±0.49 and 1.91±0.2 µg mL^{-1} IC_{50} value, respectively. The leaf extract also showed high activity in DPPH and ABTS assays. Both of the extracts possess higher activity than BHT and BHA. On the other hand, positive controls exhibited higher activity than extracts in CUPRAC assay (Figure 1).

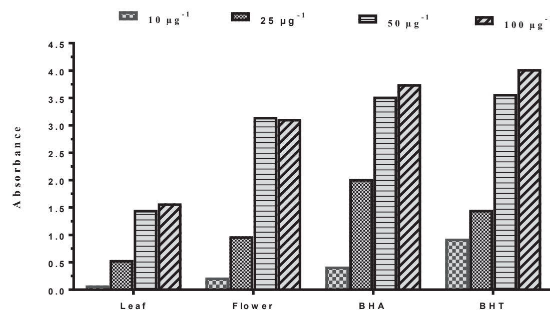

Figure 1- Cupric reducing antioxidant capacity of leaf and flower extracts of *C. syriaca* and positive controls

3.4. Mutagenic and antimutagenic activity

The methanol extracts of *C. syriaca* leaf and flower were evaluated for its mutagenic potentials towards *S. typhimurium* TA98 and TA100, both in the presence and absence of S9 mix. The different amounts of leaf extract (0.1, 0.5, 1, 5, 10, 25, 50, 100, 250, 500 µg plate^{-1}) were tested on the TA98 and TA100 strains neither any mutagenic effects were observed nor mutation frequencies significantly change when compared with spontaneous mutation frequencies (Table 3.). Flower extract increased the number of colonies at 10, 25, 50, 100, 250, 500 µg plate^{-1} concentrations in the absence of S9 mix on TA98 strain. The extract has no toxic effect at the same concentrations in the presence of S9 mix (Table 4.).

The antimutagenic experiments established in the absence of S9 mix. Results indicated that leaf extract has significant (inhibition level > 40%) antimutagenicity at 250 and 500 µg plate^{-1} concentrations (42.63% and 53.98%) on TA98 strain. On the other hand, leaf extract has moderate (inhibition level < 40-25%) antimutagenicity at 5 and 500 µg plate^{-1} concentrations (35.73% and 29.69%) on TA98 and TA100 strains respectively (Table 3.). Moreover, the flower extract showed strong antimutagenicity at 0.1 µg plate^{-1} concentration (44.17%) on TA98 strain. The flower extract has no antimutagenicity on TA100 strain (Table 4.).

According to the analysis of the leaves of *C. syriaca* which reported that it included six flavonoids (apigenin, chrysoeriol, luteolin, apigenin 7-O-glucoside, chrysoeriol 7-O-glucoside, luteolin 7-O-glucoside), four phenolic acids (caffeic acid, chlorogenic acid, 1,5-dicaffeoylquinic acid, cynarin), and three sesquiterpene lactones [11,13-dihydroxy-8-desoxygrosheimin (1) 11,13-dihydrodeacylcynaropicrin (2) solstitialin (3)] (Meriçli & Seyhan 2006).

Table 3- Mutagenic and antimutagenic activities of leaf extract of *C. syriaca*

Doses (μg plate^{-1})	TA98			TA100		
	Number of Revertants S9(-)	*Number of revertants S9(+)*	*Inhibition of Mutagenesis (%)*	*Number of Revertants S9(-)*	*Number of revertants S9(+)*	*Inhibition of mutagenesis (%)*
0	52±8	31±4	0.00	169±12	189±2	0.00
0.1	43±8	22±5	-23.61	177±15	219±4	5.63
0.5	34±5	25±3	20.55	126±13	195±19	21.48
1	50±8	20±2	-8.89	154±31	179±13	-7.99
5	46±8	23±1	35.73	126±18	183±9	-23.71
10	39±7	26±4	6.13	133±10	195±12	-20.23
50	51±2	27±5	-18.25	94±4	186±12	-21.62
100	56±3	23±3	0.46	171±12	184±10	-17.45
250	46±3	20±1	42.63	145±4	190±9	-11.89
500	60±11	23±3	53.98	122±13	177±13	29.69
DMSO	42±7	25±2	7.82	121±12	205±19	1.11
(+) Control	704±24	368±21	-	1615±45	960±30	-

(-S9) without and (+S9) with metabolic activation. Mean and ± S.D. of three plates. DMSO solvent control; 2-AF, NaN_3 and daunomycin positive control for +S9 and -S9

Table 4- Mutagenic and antimutagenic activities of flower extract of *C. syriaca*

Doses (μg plate^{-1})	TA98			TA100		
	Number of revertants S9(-)	*Number of revertants S9(+)*	*Inhibition of mutagenesis (%)*	*Number of revertants S9(-)*	*Number of revertants S9(+)*	*Inhibition of mutagenesis (%)*
0	52±8	31±4	0.00	169±12	189±2	0.00
0.1	30±5*	22±3	44.17	168±9	214±18	-0.06
0.5	33±3	24±1	0.92	161±9	175±8	3.12
1	49±5	27±2	17.63	138±13	165±7	13.00
5	343±21	29±3	-11.96	114±8	178±5	-15.50
10	nt	32±6	15.18	125±10	197±15	-1.73
50	nt	25±2	-	112±9	183±10	4.79
100	nt	28±4*	26.84	120±10	165±9	-42.55
250	nt	26±3*	32.20	126±11	176±5	-17.52
500	nt	29±2	8.43	122±8	168±9	-43.04
DMSO	42±7	25±2	7.82	121±12	205±19	1.11
(+) Control	704±24	368±21	-	1615±45	960±30	-

(-S9) without and (+S9) with metabolic activation. Mean and ± S.D. of three plates. DMSO solvent control; 2-AF, NaN_3 and daunomycin positive control for +S9 and -S9; nt, not tested; *, significantly different from the corresponding solvent control value (Dennett's test, $P<0.05$)

According to our results, it could be suggested that the antioxidant activity of the *C. syriaca* extract relates in part to its constituent both flavonoids and phenolic acids such as apigenin, aglycone, luteolin, dicaffeoylquinic acids, caffeic caffeic acid, and chrysoeriol. These constituents act as hydrogen donors, metal ion chelators, and their dividing between aqueous and lipophilic phases further influences the effectiveness.

Studies of these components, which also include *C. syriaca* extracts, have shown antioxidant and antimutagenic activities. Researches on some of these components are: Simsek & Uysal (2013) have reported the inhibitory effects of *C. cardunculus* and *C syriaca* extracts on the proliferation of human colorectal cancer DLD1 cells and inducing apoptotic pathway on DLD1 cells.

Anti-mutagenic, anti-proliferative, antioxidant, anti-inflammatory and anti-cancer activities are some activities of Apigenin. It is a flavonoid compound and found in a variety of fruits and vegetables (Madunić et al 2018). Apigenin Apigenin has shown shown potent antioxidant (Nielsen et al 1999) and strongly inhibited the bacterial mutagenesis induced by nitropyrenes (Kuo et al 1992). Luteolin 7-O-glucoside, luteolin 7-O-rutinoside and luteolin 7-O-glucuronide have shown antimutagenic effects on TA1537 and TA1535 strains (Orhan et al 2012). A number of biological activities of Apigenin 7-O-glucosid such as anticonvulsant, anti-inflammatory, antioxidant, and anticancer have been reported recently (Guzelmeric et al 2015). The pure aglycone, luteolin, have demonstrated an efficacy similar to artichoke extract in inhibiting lipid peroxidation; luteolin-7-O-glucoside, have demonstrated a dose-dependent reduction of LDL oxidation; copper chelating properties of luteolin-7-O-glucoside and luteolin have suggest a potential role for chelation in the antioxidative effects of artichoke extract (Brown & Rice-Evans 1998). Chrysoeriol have shown the ability to inhibit lipid peroxidation in low density lipoprotein induced by Cu^{2+}/O_2^- (Rice-Evans et al 1996), and antimutagenic activity in S. typhimurium TA98 (Kukić et al 2008). Chrysoeriol have found to exhibit antimutagenic activity in Salmonella typhimurium TA98 and Chrysoeriol isolated from Morinda morindoides leaves has been found to be ineffective towards superoxide radicals generated from xanthine and xanthine oxidase (Re et al 1999). Chlorogenic acid and caffeic acid have vicinal hydroxyl groups on an aromatic residue, and they exhibit antimutagenic, carcinogenic and antioxidant activities in vitro, which is to scavenge reactive oxygen species (Rice-Evans et al 1996); caffeic acid completely eliminated the mutagenicity induced by activating Glu-P-2 (2-aminodipyrido [1.2-a: 3’. 2’-d] imidazole). Both caffeic acid and chlorogenic acid effectively decreased the mutagenicity of Trp-P-I (3-amino-1,4-dimethyl-5H-pyrido-(4.3-b)indole) and Glu-P-2 (Yamada & Tomita 1996). Caffeic acid and linoleic acid has reported been that it showed inhibition on lipid peroxidation emulsion and also caffeic acid is an effective $ABTS^+$, DPPH, superoxide anion radical scavenging, total reducing power and metal chelating on ferrous ions activities (Yamada & Tomita 1996). The antioxidant activity of dicaffeoylquinic acids has been found stronger than that of ascorbic acid (Slanina et al 1999). Cynarin is a dicaffeoylquinic acid derivative of artichoke. Although cynarin is found in low amounts in the artichoke, it enhances the effect of artichoke. Choleretic and cholesterol lowering, hepatoprotective, anti-atherosclerotic, antiHIV, antioxidative, anti-diabetic, anti-carcinogenic effects are the potential healt effects of cynarin (Gezer 2017).

4. Conclusions

This study is one of the first studies in the literature investigating the antimutagenic and antioxidant activity of *C. syriaca* extract. Our findings indicate that *C. syriaca* extracts have antioxidant and antimutagenic effect. These activities of the *C. syriaca* extract may be associated, in part, with both flavonoids and phenolic acids or their synergistic effect.

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