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Cytotoxic and genotoxic effects of methanol extracts of vegetative parts of some *Gypsophila* L. species using *Allium cepa* assay

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Abstract. In this study, the cytotoxic and genotoxic effects of *Gypsophila perfoliata* L. var. *perfoliata*, *Gypsophila perfoliata* L. var. *araratica* Kit Tan, *Gypsophila pilosa* Hudson and *Gypsophila osmangaziensis* Ataşlar & Ocak plant extracts have been examined using *Allium* assay. Methanol extracts of plants have been prepared in 4 different concentrations (0.625 mg/ml, 1.250 mg/ml, 2.500 mg/ml and 5.000 mg/ml). After the onion roots were treated in these concentrations of plant extracts for 24 hours and 48 hours, mitosis slides were prepared from these root tips. With the data obtained by examining the slides, mitotic index (%) and chromosome aberration (%) values have been calculated. Distilled water has been used as the control group. It was found that mitotic index and chromosome aberration values of all species showed significant differences compared to the control group in the extract concentration range of 1.250–5.000 mg/ml. It has been also determined that the most widely observed chromosome aberrations were disturbed metaphase, sticky metaphase, c-metaphase, disturbed anaphase and anaphase bridge.

Keywords: *Gypsophila* L., Caryophyllaceae, methanol extracts, *Allium* test, mitotic index, chromosome aberrations.

INTRODUCTION

Gypsophila L., is a genus of Caryophyllaceae family, which is found between 30° and 60° latitudes and is represented with nearly 150 species in this region (Antkowiak and Dyba 2004, Schweingruber 2007). Their underground parts, containing saponins (4-25%), were used for washing wool and silk, giving halva its fragility, and as fire extinguisher agents, while in medicine they were believed to have expectorant, laxative, and emetic properties (Kołodziej *et al.* 2018).

Gypsophila which is the third largest genus of the Caryophyllaceae in Turkey, is represented by nearly 60 genus, 67% of which are endemic. Turkey

is located in *Gypsophila* species' main centers of variation and is one of the most important locations for their gene diversity (Barkoudah 1962, Bittrich 1993, Davis 1967, Davis *et al.*1988, Güner *et al.* 2000, Güner *et al.* 2012).

Studies on the saponin content of *Gypsophila* species have shown that the ratio of pure saponin can reach up to 25% in some species, for example *G. bicolor* (Freyn. & Sint.) Grossh. (Sezik 1982). Furthermore, one of the recent studies related to this subject was conducted by Kołodziej *et al.* (2018). In the study, 7 *Gypsophila* species with a potential use in the pharmaceutical industry for saponin production have been examined. The study shows that those species which were most abundant in saponins were *G. acutifolia* Steven ex Spreng., *G. pacifica* Kom., *G. scorzonerifolia* Ser. and *G. zhegulensis* Krasnova. On the other hand, Kołodziej *et al.* (2019) studied antimicrobial and antioxidant activities of the *G. paniculata* L., *G. pacifica*, and *G. scorzonerifolia*. The results of this study showed that *Gypsophila* had valuable bioactive properties and the hexane extracts showed higher antifungal and antibiotic activity. Also, Kotelnaya *et al.* (2019) mentioned plant saponins exert cardiotonic, neurotrophic, hypotensive, tonic, hypocholesterolemic and antisclerotic, diuretic, corticotropic, adaptogenic, sedative, antiulcer genic and mild laxative effects on human subjects. Natural products are potential anticancer agent sources and these are considered as alternatives to synthetic anticancer drugs which have disadvantages such as toxicity, high costs and negative side effects. It is stated that nearly 3000 plant types produce metabolites that have anticancer activity (Hartwell 1971). Furthermore, 350 plant types have been defined as potential source of agents against cancer (Graham et al. 2000). Due to the reason that modern drugs are costly, the demand for natural plant products has increased in clinical applications. Usage of plant extracts as traditional drugs in the health area is a practice which is as old as the human history. *Allium* test is an important and well known test system which is used in determining safe concentrations in the therapeutic use of plant extracts. (Roy and Roy 2019). Besides, *Allium* test is recommended as the standard test, especially for cytogenotoxicity in environmental monitoring (Fiskesjö 1985). This test has advantages such as being useful, having low costs, and showing good correlation with mammalian test systems. Results of *Allium* test are also compatible with test systems composed of prokaryotes and/or eukaryotes (Çelik and Aslantürk 2007).

In this study, the cytotoxic and genotoxic effects of methanol extracts from four *Gypsophila* species, two of which are endemic, were investigated using *Allium cepa* root tip meristem cells. The species that were chosen for this study were: *Gypsophila perfoliata* L. var. *perfoliata*, *Gypsophila perfoliata* L. var. *araratica* Kit Tan (endemic), *Gypsophila pilosa* Hudson and *Gypsophila osmangaziensis* Ataşlar & Ocak (endemic). There are some reports about cytotoxicity of different *Gypsophila* species such as *Gypsophila bicolor* (Freyn & Sint.) Grossh. and *Gypsophila ruscifolia* Boiss. (Rad *et al.* 2018).

To the best of our knowledge this is the first report on the cytotoxicity and genotoxicity of methanolic extracts of vegetative parts ofall of the studied species and we hope our research will helpshed light on other studies about *Gypsophlia* species and provide data for future studies on medicinal plants.

MATERIALS AND METHODS

Plant samples

In this study, four *Gypsophila* species were used. Plant samples were collected from two different locations. Voucher specimens were deposited at the Herbarium of the Department of Biology, Eskişehir Osmangazi University (OUFE). *G. perfoliata* var. *perfoliata* and *G. perfoliata* var. *araratica* samples were collected from B3 Afyon: Emirdağ-Çifteler junction point, steppe, 1035 m, 39°22′34.27" N and 31°02′15.21" E, 23.08.2009 (Ataşlar, OUFE 15942, OUFE 15943). On the other hand, the samples of *G. pilosa* and *G. osmangaziensis* were collected from B3 Eskişehir: Eskişehir Osmangazi University campus area, west sides, open stone areas, 810 m, 39°45′10.6164" N and 30°29′16.9620" E, 19.06.2009 (Ataşlar, OUFE 15944, OUFE 15945). These sites lie in the Central Anatolian Region which is characterized by its continental climate, extreme heat and virtually no rainfall in summers with winters recieving heavy, lasting snows. Long term avarage of annual temperature is around 10 °C while humidity is around 65% (Apaydin *et al.* 2011)

Preparation of plant extracts

The extraction procedure of the plant samples was performed according to Ataşlar *et al.* (2019). Briefly, fresh vegetative parts of the studied species were treated with 0.8% Tween 80, tap water and distilled water and then were dried at room temperature. The dried samples were grinded to obtain the powder form of the studied *Gypsophila* species. Ten grams of powdered samples were extracted with 250 ml 80% petroleum ether to remove their oily constituents with soxhlet apparatus. The degreased plant materials were dried overnight and were extracted with 250 ml 70% methanol. At this step, the flasks were mixed for half an hour in the blender

consecutively three times. All of the methanol extracts were combined and filtrated with Whatman 1 filter paper. The methanol in the total extract was removed by rotary evaporator. The obtained dry extracts (yield= 6.90%, 5.00%, 8.96% and 7.36%, respectively) were maintained at 4 °C for future use in genotoxicity studies.

Genotoxicity Test

Genotoxicities of plant extracts used in the study have been determined with *Allium* Test. For this purpose, the methanol extracts of *G. perfoliata* var. *perfoliata*, *G. perfoliata* var. *araratica*, *G. pilosa* and *G. osmangaziensis* species were prepared in 4 different concentrations (0.625 mg/ml, 1.250 mg/ml, 2.500 mg/ml and 5.000 mg/ml). Distilled water has been used as negative control. For each concentration, 6 onions rooted in distilled water for 24 hours. Onion roots were left to interact with extract concentrations at 25 ± 1 °C for 24-48 hours. At the end of 24 and 48 hours, the root tips were cut and included in the Farmer fixative (3: 1 ethyl alcohol: glacial acetic acid) and stored at +4 °C. Fixative residues that could be found on the root tips were removed by washing with distilled water. Afterwards, the root tips have been hydrolyzed for 12 minutes in 1 N HCl acid at 60 °C water bath. After the hydrolysis, the roots were submerged in Feulgen dye and chromosomes were stained for 1 hour with the Schiff reaction. After this procedure, slides were prepared from the dyed root tips (1-2 mm) by crushing and spreading. (Fiskesjö 1985, Rank *et al.* 2002, Rank 2003).

Slides were phtographed using a light microscope, Nikon Eclips 80*i*. Mitotic Index % (MI%). Mitotic Index (MI%) have been calculated with the following formula (Sehgal et al. 2006) and Chromosome Aberration % (CA%) have been calculated with the other formula (Ivanova et al. 2003).

Mitotic Index (MI) (%)=(P+M+A+T)/(Total number of cells)×100

Chromosome Aberration (CA) (%)=(Number of abnormal cells)/(Number of cells in mitosis)×10

where (P+M+A+T) is the sum of all cells in phase as prophase, metaphase, anaphase and telophase, respectively.

Statistical Analysis

The results have been interpreted statistically, using independent sampling T test, one-way variance analysis (ANOVA) and Tukey test.

RESULTS AND DISCUSSION

The values of MI% (Mitotic Index %) and CA% (Chromosome Aberration %) of the methanol extracts of *Gypsophila* species used in the study are given in Table 1. In the 24-hour treatment, 5.000 mg / ml concentrationss significantly decreased MI% in *G. osmangiensis* and *G. pilosa* extracts compared to the control group ($P<0.05$). MI% values have statistically decreased to a significant extent compared to the control group, at 2.500 mg/ml and 5.000 mg/ml concentrations of *G. perfoliata* var. *perfoliata* extract and at 1.250 mg/ml, 2.500 mg/ml and 5.000 mg/ml concentrationsof *G. perfoliata* var. *araratica* extract ($P<0.05$). As a result of treatment for 48 hours, none of the MI% values relating to *G. osmangaziensis* extract showed a significant difference compared to the control group. For *G. pilosa* extract, 0.625 mg/ml, 1.250 mg/ml concentrations have increased MI% compared to the control group, whereas 2.500 mg/ml has reduced it. When MI% values of *G. perfoliata* var. *perfoliata* have been considered, it was determined that only 5.000 mg/ml has shown a significant decrease compared to the control group and for *G. perfoliata* var. *araratica* extract, it was determined that 2.500 mg/ml and 5.000 mg/ml concentrations have shown a significant decrease compared to the control group ($P<0.05$).

The mitotic index is a cytogenetic parameter that helps measure the proliferation (M phase) of mitotic cells in the cell cycle, and inhibition of the mitotic index is considered as cell death. (Öney-Birol and Gündüz 2019). Taking this into consideration, the result of the treatment for 24 hours in the *G. Osmangaziensis* extract which had a concentration of 5.000 mg/ml, MI% decreased to a significant extent, which means that it showed a cytotoxic effect. However, no such effect could be observed from 48 hours treatment in the same concentration for 48 hours. In the case of *G. Pilosa*, in the samples that were treated for 24 hours, the 5.000 mg/ml concentration decreased MI% and in the samples that were treated for 48 hours, 2.500 mg/ml concentration decreased MI%, meanwhile 1.250 mg/ml and 0.625 mg/ml concentrations increased MI%. When we look at the experiment data of the *G. perfoliata var. perfoliata* extract samples, it is seen that after 24 hours treatment the 2.500 mg/ml and the 5.000 mg/ml concentrations decreased MI% meanwhile in the samples that were treated for 48 hours only the 5.000 mg/ml concentration decreased MI%. The treatment of samples in *G. perfoliata var. araratica* for 24 hours showed a decrease in MI% in 1.250 mg/ml, 2.500 mg/ml and 5.000 mg/ml concentrations while treatment for 48 hours only showed a decrease in MI% in 2.500 mg/ml and 5.000 mg/ml

Table 1. Mitotic index and chromosome aberration types and their frequency induced by *Gypsophila* extracts in root tip cells of *Allium cepa*.

Duration	Plant species	Concentration of treatment (mg/ml)	Total number of cells scored	Number of dividing cells	Number of abnormal cells	Mitotic index (%)±SD	Chromosome aberration types (%)					The frequency of total chromosome aberration (CA %)±SD
							Disturbed metaphase	Sticky metaphase	c-metaphase	Disturbed anaphase	Anaphase bridge	
"24 hours	*G. osmangaziensis*	Control	12959	740	21	5.76±2.69	43	48	0	9	0	2.65±1.05
		0.625	13498	902	29	6.72±1.40	45	38	3	3	11	3.25±085
		1.250	12704	966	53	7.69±1.88	43	30	4	17	7	5.50±1.41 [b]
		2.500	12740	739	46	5.81±1.19	54	31	0	13	2	6.16±1.49 [b]
		5.000	11916	364	30	2.90±1.25[a]	53	43	0	4	0	6.09±4.71 [b]
	G. pilosa	Control	11094	588	0	5.41±1.02	0	0	0	0	0	0.00±0.00
		0.625	12763	636	0	5.06±0.75	0	0	0	0	0	0.00±0.00
		1.250	12698	648	0	5.18±0.81	0	0	0	0	0	0.00±0.00
		2.500	11397	511	30	4.44±1.35	43	27	0	27	3	6.28±2.53 [b]
		5.000	11827	231	28	2.02±1.29 [a]	39	29	0	32	0	14.15±10.97 [b]
	G. perfoliata var. *perfoliata*	Control	13001	687	0	5.43±1.47	0	0	0	0	0	0.00±0.00
		0.625	12756	905	0	7.11±1.24	0	0	0	0	0	0.00±0.00
		1.250	12202	695	0	5.70±0.60	0	0	0	0	0	0.00±0.00
		2.500	13271	389	19	2.86±1.22 [a]	63	27	0	5	5	5.51±5.52 [b]
		5.000	10574	281	16	2.61±1.10 [a]	13	50	6	31	0	6.09±4.26 [b]
	G. perfoliata var. *araratica*	Control	10753	849	0	7.83±1.39	0	0	0	0	0	0.00±0.00
		0.625	11338	820	0	7.38±1.88	0	0	0	0	0	0.00±0.00
		1.250	12230	689	13	5.85±1.68 [a]	38	31	8	23	0	2.05±1.64 [b]
		2.500	12744	609	23	4.80±0.87 [a]	48	30	0	13	9	3.88±2.18 [b]
		5.000	11810	337	37	2.92±1.02 [a]	54	16	3	27	0	10.61±5.39 [b]
48 hours	*G. osmangaziensis*	Control	12243	550	1	4.29±2.82	100	0	0	0	0	1.19±2.92
		0.625	12628	612	27	4.88±1.47	52	33	0	11	4	3.78±3.23
		1.250	12149	520	45	4.28±1.07	20	31	0	49	0	8.60±7.62 [b]
		2.500	12429	558	15	4.53±1.75	20	53	0	27	0	3.19±2.82
		5.000	13159	502	13	3.84±1.39	31	69	0	0	0	2.44±2.07
	G. pilosa	Control	12904	661	0	5.22±0.77	0	0	0	0	0	0.00±0.00
		0.625	11542	811	0	7.07±0.78 [b]	0	0	0	0	0	0.00±0.00
		1.250	10919	587	0	5.28±1.25 [b]	0	0	0	0	0	0.00±0.00
		2.500	10854	340	15	3.13±1.30 [a]	40	47	0	13	0	5.11±3.02 [b]
		5.000	11708	238	12	1.97±1.23	61	23	8	8	0	4.48±2.68 [b]
	G. perfoliata var. *perfoliata*	Control	14266	726	0	5.11±1.22	0	0	0	0	0	0,00±0,00
		0.625	12969	827	0	6.39±1.00	0	0	0	0	0	0.00±0.00
		1.250	11701	704	0	5.99±0.71	0	0	0	0	0	0.00±0.00
		2.500	12947	520	33	4.10±1.99	49	42	0	9	0	5.43±3.70 [b]
		5.000	11895	155	16	1.26±0.70 [a]	31	38	6	25	0	9.44±6.02 [b]
	G. perfoliata var. *araratica*	Control	10731	675	0	6.15±1.85	0	0	0	0	0	0.00±000
		0.625	12499	732	0	5.94±0.82	0	0	0	0	0	0.00±0.00
		1.250	12960	793	22	6.11±0.69	18	59	0	23	0	2.76±1.50 [b]
		2.500	12507	523	29	4.28±0.83 [a]	48	28	3	21	0	5.56±3.84 [b]
		5.000	12121	362	33	2.94±1.03 [a]	52	36	0	12	0	10.95±6.51 [b]

Means in a column followed by the same superscript letters are significantly different according to their control groups (P<0.05, one-way ANOVA, Tukey post hoc test; a: reduction of MI and CA, b: increase in MI and CA).

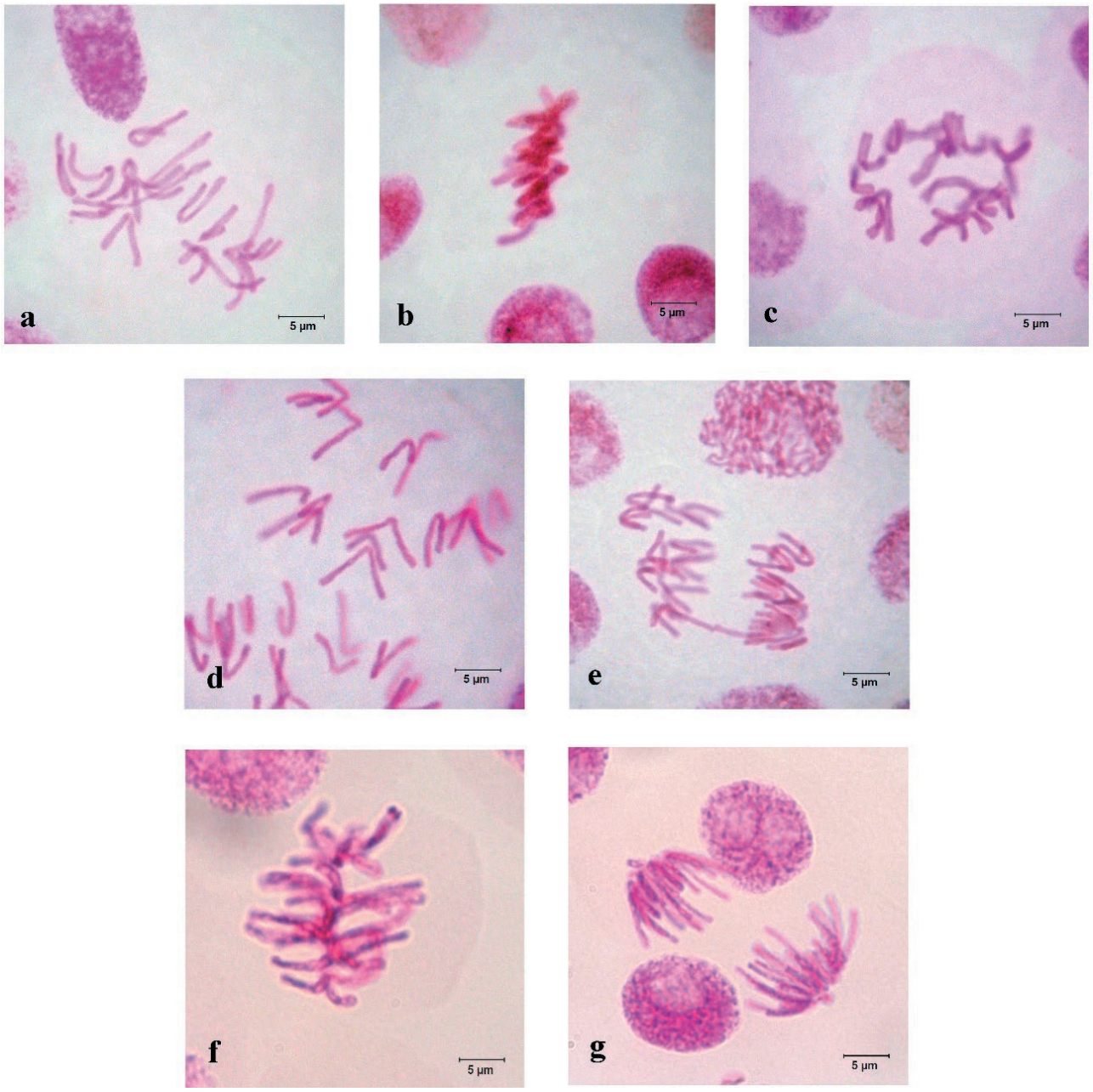

Figure 1. Chromosome aberrations in *Allium cepa* root meristem cells after treatment with extracts of *Gypsophila* species. **a:** Disturbed metaphase (*G. perfoliata* var. *araratica*-24 hours-5.000 mg/ml); **b:** Sticky metaphase (*G. pilosa*-24 hours-2.500 mg/ml); **c:** c-metaphase (*G. perfoliata* var. *araratica*-24 hour-5.000 mg/ml); **d:** Disturbed anaphase (*G. perfoliata* var. *perfoliata*-48 hours-5.000 mg/ml); **e:** Anaphase bridge (*G.osmangaziensis*-24 hours-2.500 mg/ml);**f:** normal metaphase and; **g:** normal anaphase.

concentrations. Thus, it can be said that at the end of 48 hours a decrease in the cytotoxicity of these plant extracts is observed.

When reviewing the literature, it can be seen that *Caryophyllaceae,* which the studied plant species are included, constitute a wide family that has cytotoxic species. Cytotoxic activity of *G. bicolor* and *G. ruscifolia*'s methanol extracts on MCF-7 (human breast adenocarcinoma), A-549 (non-small cell lung carcinoma) and AGO1522 (human fibroblast) cell lines were examined using MTT method and it was determined to be cytotoxic for MCF-7 (human breast adenocarcinoma) cells

(Rad *et al*. 2018). In another study, it was determined that *Gypsophila* saponins showed a synergistic cytotoxicity in macrophage-like (PMA-treated) U937 cells with type I RIPs saporin and his-tagged saporin (Weng *et al.* 2008).

In a study conducted by Gevrenova *et al.* (2014), it was determined that saponins were plant glycosides having one or more sugar chains being covalently linked as a steroid or triterpenoid aglycon or aglycon and it was emphasized that *Caryophyllaceae* was an extremely rich source of triterpene saponin. In this study, it was shown for the first time that extracts of *Caryophyllaceae* species including *Saponaria officinalis* L., *Gypsophila trichotoma* Wend. and *Dianthus sylvestris* Wulffen, had impact on the vitality of mammalian monocytes/macrophage cell lines and that they induced apoptosis through caspase-3 activation (Gevrenova *et al.* 2014). In the literature research that has been made, no study was found using the *Allium* Test in determining the cytotoxicity of plant extracts belonging to *Gypsophila* species.

Another parameter determined in this studyis genotoxicity. For this purpose, CA% values were calculated. These values are shown in Table 1. In the chromosome analysis of the four *Gypsophila* species being studied, aberrations in the form of disturbed metaphase, sticky metaphase, c-metaphase, disturbed anaphase and anaphase bridge were observed. These aberrations are shown in Figure 1. Similar results were observed in the literature. Ždralović and colleagues found that methanol extracts of the *Plantago lanceolata* L. plant also caused chromosome aberrations like sticky metaphase and anaphase bridge in *Allium cepa* chromosomes (Ždralović *et al*. 2019).

The deterioration of microtubules frequently causes mitotic aberrations like laggard chromosomes resulted from disturbed anaphase-telophase (Amer and Ali, 1986). Various abnormalities like lagging chromosomes, vagrants, distrubed metaphases and anaphases, and chromosome stickiness can be induced by the inhibition of proteins' effect on the spindle function (Tkalec *et al.* 2009). And chromosome stickiness, usually of an irreversible type leading to cell death, is definite proof of genotoxicity (Khanna, N., & Sharma, S. (2013). Moreover, vagrant chromosomes and c-metaphases increase the risk for aneuploidy, whereas chromosome bridges indicate the clastogenic effect caused by chromosome breaks (Leme and Marin-Morales 2009).

When plant extracts' genotoxicity was examined, distilled water was used as negative control. In the 24 hours treatment, it was seen that CA% values of *G. osmangaziensis* extract with concentrations of 1.250 mg/ml and 2.500 mg/ml 5.000 mg/ml increased significantly compared to the control group. For *G. pilosa* extract, concentrations of 2.500 mg/ml and 5.000 mg/ml increased CA%. For *G. perfoliata* var. *perfoliata,* concentrations of 2.500 mg/ml and 5.000 mg/ml increased CA% significantly compared to the control group and for *G. perfoliata* var. *araratica,* concentrations of 1.250 mg/ml, 2.500 mg/ml and 5.000 mg/ml increased CA% significantly compared to the control group ($P<0.05$). As a result of treatment for 48 hours, it was seen that for *G. osmangaziensis* extract, only the concentrationof 1.250 mg/ml increased CA% statistically and that for *G. pilosa,* concentrations of 2.500 mg/ml and 5.000 mg/ml statistically increased the CA% value. For *G. perfoliata* var. *perfoliata,* concentrations of 2.500 mg/ml and 5.000 mg/ml increased CA% values and for *G. perfoliata* var. *araratica,* concentrations of 1.250 mg/ml, 2.500 mg/ml and 5.000 mg/ml increased CA values. According to these results; after 24 hour treatment, only the 1250 mg/ml, 2250 mg/ml and 5000 mg/ml concentrations of G. Osmangaziensis extract and after 48 hour treatment, only the 1250 mg/ml concentration of the same extract exhibited a significant increase. For the other 3 plant extracts; a significant difference in CA% increase between the 24 hour and 48 hour treatments could not be observed. Thus it can be said that treatment period does not effect genetoxicity to a great extent concentration.

In the literature review that has beenmade, no other genotoxicity study was found on these plant extracts. The fact that *G. Osmangaziensis* is a new specie (Ataşlar and Ocak 2005), emphasizes the importance of data submitted in this study. However, it is considered appropriate for *in vitro* tests such as this to be evaluated with other test systems. Our future studies will aimto support the data in this study related to the species investigated with other in vitro test systems.

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