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Genotoxic and mutagenic effects of aqueous extract from aerial parts of *Achillea teretifolia*

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Abstract: Genotoxic and mutagenic effects of aqueous extract from aerial parts of *Achillea teretifolia* (*At*-ext) were investigated using both *Allium cepa* root meristematic cells and a short-term mutation assay in *Salmonella typhimurium* with TA98 and TA100 strains in the presence or absence of S9 mix, respectively. In the *Allium* tests, EC_{50} value was determined as 50 g/L, and 0.5 × EC_{50}, EC_{50}, and 2 × EC_{50} concentrations of *At*-ext were applied to onion tuber roots. Methyl methane sulfonate (MMS, 10 ppm) and distilled water were used as positive and negative controls, respectively. The mitotic index (MI) increased in all concentrations in comparison to control at each exposure time except 25 and 50 g/L at 12 h. While stickiness, chromosome laggards, bridges, and disturbed anaphase-telophase were observed in anaphase-telophase cells, pro-metaphase, c-metaphase, polyploidy, and binuclear cells were observed in other cells. It was determined that *At*-ext have no mutagenic effect on *S. typhimurium* TA98 and TA100 strains in the presence or absence of S9 mix. The results were statistically analyzed in SPSS using Duncan's multiple range test. These results indicate that *At*-ext has genotoxic activity in *A. cepa* root meristematic cells.

Key words: *Allium,* Ames, chromosome aberration, mutagenicity

Introduction

The genus *Achillea* (Asteraceae) was named after Achilles, a mythological warrior who used this plant to heal wounds. This genus comprises approximately 85 species around the world, most of them endemic. *Achillea* L. has 42 species in Turkish flora, and 23 of them are endemics in Turkey (1-3). Some of these, especially *A. millefolium*, are used in traditional medicine for several purposes such as curing diarrhea, abdominal pain, and stomachache due to the lactones, achillein, and azulene. It is also used to treat hemorrhoids and wounds (diuretic, emmenagogue agents), and possesses other medicinal components (4-11). In spite of the curative implementations of *Achillea*, it produces toxic effects in human beings such as asthma, contact dermatitis, and rhinitis (12-14).

Numerous plants such as *Crepis capillaris*, *Tradescantia paludosa*, *Vicia faba*, *Hordeum vulgare*, *Pisum sativum*, and *Allium cepa* have been used to determine the mutagenic and cytogenetic effects of chemicals. The *Allium* test is one of the most reliable test systems for determining toxicity in vitro (15-20). Since the onions are easy to stock and treat, observation of their microscopic and macroscopic variables can be easily performed. In addition, this test is mutually related to data obtained from both prokaryotic and eukaryotic organisms (21).

When examining the genotoxicity of plant extracts or chemical(s), the *Salmonella*/microsome test (Ames test) is commonly used (22-24). This test can be carried out rapidly and cheaply; it is also one of the most dependable short-run bacterial test systems (25,26). Mutant strains of *S. typhimurium*, obtained

from *S. typhimurium* LT2 parental line in vitro, are used in this test system (27).

The aim of the present study was to examine the genotoxic and mutagenic effects of *At*-ext by employing a bacterial reverse mutation assay in *S. typhimurium* TA98 and TA100 strains with or without S9 mix and the *Allium* test, respectively.

Materials and methods

Organisms

The *S. typhimurium* test strains TA98 and TA100 were kindly supplied by Prof Nuran Diril, of Hacettepe University, Turkey. TA100 was used for establishing the base pair exchange and TA98 for defining the frame shift type of mutations.

Allium cepa (2n = 16) onion bulbs, 25-30 mm diameter, and without any treatment, were purchased from a local supermarket.

Chemicals

Bacto agar, S9 mix from rat liver (Sprague-Dawley), β-nicotinamide-adenine dinucleotide phosphate (β-NADP), nutrient broth no. 2 (Oxoid), ampicillin, tetracycline, glucose-6-phosphate (G6P), 2-aminoanthracene (2AA), histidine, and basic fuchsine were purchased from Sigma-Aldrich. NaOH, KCl, NaCl, citric acid monohydrate, and sodium azide (SA) were purchased from Riedel. Fluka supplied 2-aminofluorene (2AF) and 4-Nitro-o-phenylenediamine (NPD). The remaining chemicals used in the study were obtained from Merck.

Plant collection and extraction

The aerial parts of *A. teretifolia* were collected from Kumalar Mountain (located in the transitional zone of the Aegean-Central Anatolia regions, Afyonkarahisar, Turkey, ~1400-1500 m) in late July 2010. The taxonomic identification of plant materials was confirmed by Dr M Kargıoğlu, Biology Department, Afyon Kocatepe University, Turkey. A voucher specimen is kept at the Afyon Kocatepe University Herbarium. Air-dried and pulverized aerial parts of the *A. teretifolia* were used in the extracting procedure. To 1 L of boiling water was added 100 g of plant specimen over 10 min (28). Both the squeezed extracts and decoctions were filtered using a 2.5 μm filter (Whatman® no. 42) to eliminate the remaining particles. The extract was then dried down in vacuo by rotary evaporator. The residue was dissolved in 10 mL of distilled water and kept at 4 °C. These were the stock solutions, and fresh extracts were prepared daily in all applications.

Allium test

Before initiating the test, both thin outer skins of the bulbs and the dry bottom portion were removed without destroying the primordial roots. In determining the effective concentration (EC_{50}), a series of 6 bulbs were placed in distilled water for 24 h. Subsequently, the 5 best-growing bulbs were introduced into the *A. teretifolia* solutions (10, 20, 40, 60, and 80 g/L, respectively) at room temperature (~21 ± 4 °C) for 4 days. The investigation concentrations were refreshed every 24 h during the tests. On the fifth day, root lengths (10 roots from each bulb) were measured from both *At*-ext exposed and control bulbs. EC_{50} value was the concentration that decelerates root growth by 50% in comparison to control.

In determining application doses, 2 × EC_{50}, EC_{50}, and 0.5 × EC_{50} negative and positive (MMS, 10 ppm) control groups were used at 12, 24, 48, 72, and 96 h. The root tip cells were fixed and stained according to earlier methods (24,29). The root tips were fixed in Carnoy's fixative and stained with Feulgen stain for 1 h. In determining chromosomal aberration (CA) frequencies and MI, the method of Saxena et al. (18) was followed. For each test group, 5 slides (1 root tip/slide) were prepared by mashing the root tips in 45% acetic acid. Slides were then randomly coded and scored blindly. In determining MI, different mitotic stages were counted in at least 5000 cells (1000 cells/slide) per concentration and expressed as a percentage. To determine CAs, 100 cells in anaphase or telophase were checked for aberrations (per slide when possible).

Ames plate incorporation test

The cytotoxic doses of *At*-ext (10,000, 1000, 100, 10, 1, and 0.1 μg/plate) were determined according to Dean et al. (30).

The Ames test was carried out as a standard plate incorporation test with TA98 and TA100 strains of *S. typhimurium* in the presence or absence of S9 mix

(25). The strains were selected according to Dean et al. (30), and then examined according to the related genetic markers. For each tester strain, a specific positive control was always used to test the experimental defects, if any. SA for TA100 and NPD for TA 98 were used as positive controls in the absence of S9 mix. Both 2AA and AF were also used as positive controls in the presence of S9 mix.

For each concentration 100 µL of the test solution, 500 µL of S9 mix mix (or 500 µL phosphate buffer), and 100 µL of cell suspension from an overnight culture (1-2 × 10^9 cells/mL) were added to 2 mL top agar (kept at 45 °C) and vortexed for 3 s. The entire mixture was covered on the minimal agar plate, and the plates were incubated at 37 °C for 72 h. Following the incubation period, the revertant bacterial colonies on each plate were counted. Both positive and negative controls (distilled water) were also preserved concurrently. Samples on triplicate plates in 2 independent parallel experiments were examined.

Statistical analysis

The data obtained for root length, MI, mitotic phases, and CA were expressed as percentages. The levels of difference in treatment groups were analyzed statistically by SPSS 15.0 version for Windows. In the analyses, Duncan's multiple range test was performed by ANOVA on both the *Allium* and Ames tests.

Results and discussion

Results of the *Allium* root growth test are shown in Table 1. The EC_{50} was 50 g/L. These results showed that the effect of *At*-ext on *Allium* root growth was dose-dependent. Root growth increased up to 40 g/L concentration; at the higher (40 g/L), it decreased. It was previously reported that root growth inhibition is generally related to apical meristematic activity (31) and cell elongation during differentiation (32). The occurrence of stunted roots is an indicator of both retardation of growth and cytotoxicity (29). In concentrations greater than 40 g/L roots became dark colored, thicker, gel-like formations. These results support the contention that *At*-ext is necessary for growth but that the line between useful and harmful concentrations is very thin.

Table 1. Results of the *Allium* root growth inhibition test.

Doses (g/L)	Average length (cm) ± SD*	Growth (%)	Increase (+) or decrease (–) in growth (%)
Control	2.59 ± 0.63a	100	0.00
10	3.19 ± 0.38b	123.05	+ 23.05
20	3.05 ± 0.43b	117.61	+ 17.61
40	1.50 ± 0.36c	57.80	−42.20
60	1.11 ± 0.36d	42.58	−57.42
80	0.96 ± 0.37d	36.99	−63.01

*: different letters differ significantly at the level of 0.05; SD: standard deviation.

Table 2 summarizes the effect of *At*-ext on MI and mitotic phase in the root meristematic cells of *A. cepa* treated for 12, 24, 48, 72, and 96 h. In the root incubations, while all applied concentrations increased MI, MMS decreased MI when compared to the negative control at each exposure time. The difference between increased and decreased MIs was statistically significant ($P < 0.05$), and the effect on MI was dose-dependent. An increase or decrease in levels of MI can be an indicator of the cytotoxicity of the agent examined (33). Lower MIs of exposed organisms in negative control may indicate the toxic effect of test compounds. In contrast, MIs higher than those found in negative control may result from cell division induction. This might be characterized by a condition harmful to cells, which could lead to uncontrolled proliferation and tumor formation (34). Increased cell proliferation could be a result of time shortage for DNA repair (35). All applied concentrations of *At*-ext caused alterations in the proportions of distribution of particular mitotic phases compared to control. The characteristic effect of the tested preparations was an increase in the prophase index and simultaneous decreases in the metaphase and anaphase indexes except for the 25 g/L concentration at 24 h. The telophase index decreased as well, and this was a dose-dependent effect. This may be due to chfr point (control point between prophase/metaphase) blockage. Scolnicand and Halazonetis (36) stated that chfr specifies a

Table 2. The effects of *At*-ext on MI and mitotic phase of *A. cepa* root meristem cells.

Concentration (g/L)	Counting cell number	MI ± SE*	Mitotic phases (%) ± SE*			
			Prophase	Metaphase	Anaphase	Telophase
Control-12 h	5078	36.08 ± 0.54a	69.55 ± 2.91a	12.88 ± 0.83a	11.61 ± 2.83a	5.96 ± 1.39a
MMS-10 ppm	5131	25.95 ± 3.49b	96.39 ± 0.70b	2.21 ± 0.08b	0.89 ± 0.05b	0.51 ± 0.06b
25	5037	41.52 ± 0.74c	80.24 ± 4.52c	10.10 ± 1.05c	6.28 ± 2.97ab	3.38 ± 0.69c
50	5138	44.37 ± 0.72d	93.08 ± 0.65b	4.23 ± 0.46b	0.93 ± 0.21b	1.76 ± 0.62bc
100	5109	56.92 ± 1.75e	91.15 ± 0.98b	6.96 ± 1.85d	1.35 ± 0.35b	0.54 ± 0.11b
Control-24 h	5136	35.71 ± 0.60a	84.21 ± 2.15a	5.83 ± 0.85a	3.45 ± 0.50a	6.51 ± 1.07a
MMS-10 ppm	5114	25.55 ± 0.84b	89.38 ± 1.32b	5.85 ± 0.47a	3.52 ± 0.60a	1.25 ± 0.27b
25	5122	44.88 ± 0.61c	82.01 ± 2.32a	6.68 ± 0.71a	5.24 ± 0.67b	6.07 ± 1.17a
50	5190	53.59 ± 0.40d	94.30 ± 0.19c	3.48 ± 0.12b	1.57 ± 0.09c	0.65 ± 0.07b
100	5062	58.36 ± 0.55e	96.80 ± 0.33c	2.70 ± 0.13b	0.27 ± 0.10c	0.23 ± 0.06b
Control-48	5200	36.57 ± 0.32a	85.70 ± 1.21a	6.25 ± 0.34a	4.50 ± 0.39a	3.55 ± 0.58a
MMS-10 ppm	5252	30.08 ± 1.41b	84.68 ± 0.53a	8.88 ± 0.32b	5.25 ± 0.42a	1.19 ± 0.19b
25	5186	45.07 ± 0.49c	91.54 ± 0.78b	4.79 ± 0.33c	2.52 ± 0.28b	1.15 ± 0.24b
50	5164	55.10 ± 0.60d	95.76 ± 0.30c	2.38 ± 0.29d	1.16 ± 0.05c	0.70 ± 0.07b
100	5138	58.34 ± 0.60e	94.79 ± 0.55c	4.12 ± 0.55c	0.79 ± 0.15c	0.30 ± 0.08b
Control-72	5244	34.38 ± 0.94a	84.42 ± 0.77a	7.22 ± 0.39a	5.24 ± 0.32a	3.12 ± 0.26a
MMS-10 ppm	5027	29.30 ± 1b	81.29 ± 2.19b	11.23 ± 1.03b	4.54 ± 0.81a	2.94 ± 0.48a
25	5168	46.05 ± 0.89c	87.86 ± 1.12a	6.52 ± 0.64ac	4.03 ± 0.24a	1.58 ± 0.29b
50	5220	55.89 ± 0.64d	93.16 ± 0.38c	5.54 ± 0.18c	1.27 ± 0.15b	0.42 ± 0.13c
100	5203	59.78 ± 0.34e	93.01 ± 0.45c	5.31 ± 0.26c	1.49 ± 0.19b	0.19 ± 0.03c
Control-96	5102	39.97 ± 0.40a	82.14 ± 0.33a	8.84 ± 0.55a	5.22 ± 0.32a	3.80 ± 0.18a
MMS-10 ppm	5176	24.90 ± 1.08b	76.64 ± 1.76b	11.37 ± 0.66b	7.66 ± 1.18b	4.33 ± 0.16b
25	5213	41.32 ± 0.62c	87.44 ± 0.88c	7.57 ± 0.62a	3.17 ± 0.30c	1.84 ± 0.13c
50	5115	54.95 ± 0.86d	95.65 ± 0.53d	3.54 ± 0.49c	0.56 ± 0.09d	0.25 ± 0.04d
100	5124	61.46 ± 2.01e	98.13 ± 0.11d	1.49 ± 0.06d	0.29 ± 0.06d	0.09 ± 0.04d

*: different letters differ significantly at the level of 0.05; SE: standard error.

checkpoint that delays cell entry into metaphase. The increased and decreased phase indexes differed significantly ($P < 0.05$) except for the 25 g/L application at 24 h.

In Table 3, the results of the *A. cepa* anaphase-telophase chromosome aberration test are shown. The most frequent abnormalities were anaphase bridges, chromosome laggards, disturbed anaphase-telophase, and stickiness. The effects of different concentrations of *At*-ext on CA were significant ($P < 0.05$). They were also dose-dependent for all tested concentrations at 48, 72, and 96 h compared to negative control. In addition to the abnormalities mentioned above, pro-metaphase, polyploidy, c-metaphase, and binuclear cells were observed. These results were statistically significant ($P < 0.05$) when compared to other

Table 3. Percentage of chromosome aberrations of *At*-ext in different times and concentrations obtained for the *Allium* test.

Concentration	Anaphase-telophase anomalies (%)						Other anomalies (%)					
(g/L)	CCN	S	AB	CL	DAT	TA ± SE*	CCN	CM	PM	P	BNC	TA ± SE*
Control-12 h	500	4	4	6	7.20	**21.20 ± 2.26a**	5078	0.37	0.38	0.28	0.6	**1.08 ± 0.11a**
MMS-10 ppm	250	17.27	10.30	13.11	26.74	**67.42 ± 10.86b**	5131	0.21	0.35	-	-	**0.56 ± 0.04a**
25	418	1.67	1.20	5.02	8.13	**16.06 ± 0.23a**	5037	0.40	0.72	0.36	0.26	**1.74 ± 0.87b**
50	340	2.35	1.18	2.06	4.12	**9.79 ± 0.98a**	5138	0.12	0.92	0.18	0.55	**1.77 ± 0.12b**
100	142	4.93	4.23	12.68	16.2	**38.35 ± 3.26c**	5109	0.42	1.95	0.39	0.29	**2.99 ± 0.60c**
Control-24 h	500	2.40	2	4.40	5.80	**14.60 ± 1.24a**	5136	0.16	2.29	0.08	0.20	**1.67 ± 0.11a**
MMS-10 ppm	291	4.41	3.48	5.47	6.28	**19.63 ± 1a**	5114	0.29	0.63	0.16	0.08	**1.15 ± 0.08b**
25	500	2.40	3.20	8	7.60	**21.20 ± 0.96a**	5122	0.35	1	0.14	0.08	**1.56 ± 0.09a**
50	222	4.55	2.51	4.34	8.88	**20.27 ± 2.10a**	5190	0.31	1.25	0.14	0.04	**1.73 ± 0.10a**
100	74	2.26	2.36	8.12	5.12	**17.95 ± 3.54a**	5062	0.16	1.42	0.08	0.20	**1.86 ± 0.23a**
Control-48	500	1.80	2.80	3.20	4	**11.80 ± 0.73a**	5200	0.46	0.58	0.12	0.15	**1.30 ± 0.17a**
MMS-10 ppm	286	3.76	3.28	9.66	9.17	**25.86 ± 0.59b**	5252	0.53	0.65	0.13	0.17	**1.48 ± 0.05a**
25	386	4.90	4.90	13.96	12.16	**35.91 ± 1.34c**	5186	0.80	1.14	0.24	0.17	**2.34 ± 0.03bc**
50	206	3.88	8.25	9.37	16.39	**37.89 ± 0.69c**	5164	0.68	1.53	0.21	0.15	**2.57 ± 0.07c**
100	66	5.84	14.79	26.17	30.70	**75.51 ± 3.89d**	5138	0.49	1.41	0.15	0.05	**2.09 ± 0.19b**
Control-72	500	4.20	4	5	5.20	**18.40 ± 1.72a**	5244	0.50	0.74	0.08	0.13	**1.45 ± 0.09a**
MMS-10 ppm	299	14.95	6.89	10.39	10.40	**42.62 ± 2.41b**	5027	0.69	0.85	0.16	0.06	**1.76 ± 0.10a**
25	362	5.75	7.97	14.87	14.17	**45.72 ± 1.54b**	5168	0.91	1.29	0.25	0.25	**2.70 ± 0.19b**
50	156	4.89	9.19	14.17	18.71	**46.96 ± 2.09b**	5220	0.82	1.88	0.29	0.40	**3.39 ± 0.15c**
100	81	10.17	28.76	18.80	24.84	**82.56 ± 2.69c**	5203	1.90	2.11	0.13	1.63	**4.47 ± 0.18d**
Control-96	500	4.2	4	5.20	6.40	**19.80 ± 3.1a**	5102	0.67	0.61	0.10	0.06	**1.42 ± 0.11a**
MMS-10 ppm	320	18.09	9.19	12.43	12.67	**52.37 ± 2b**	5176	1.88	2.11	0.27	0.15	**4.40 ± 0.27b**
25	390	14.58	5.65	13.09	17.20	**50.51 ± 1.53b**	5213	1.13	1.26	0.08	0.06	**2.52 ± 0.05c**
50	160	21.94	16.90	15.7	13.78	**68.32 ± 7.01c**	5115	0.72	1.35	0.19	0.02	**2.28 ± 0.26c**
100	62	32.51	11.14	16.84	17.46	**77.96 ± 2.47c**	5124	1.54	1.78	0.08	-	**3.39 ± 0.10d**

*: letters differ significantly at the level of 0.05; SE: standard error; CCN: counting cell numbers; S: stickiness; AB: anaphase bridge; CL: chromosome laggards; DAT: disturbed anaphase-telophase; TA: total anomalies; PM: pro-metaphase; CM: c-metaphase; P: polyploidy; BNC: binuclear cell.

anomalies. Stickiness (especially at 100 g/L at 96 h) is an indicator of an irreversible, highly toxic effect of *At*-ext. Its occurrence in the study might be due to a sub-chromatid linkage between chromosomes (37). Anaphase bridges may occur due to the translocation of the unequal chromatid exchange, breakage and fusion of chromosomes and chromatids, or dicentric chromosome presence. These bridges are one of the causes of structural chromosome mutations (38). Disturbed anaphase-telophase and chromosome laggards may develop due to the microtubule malformation effect of *At*-ext (39). The occurrence of chromosome laggards at anaphase is dependent on the failure of chromosomes or acentric chromosome fragments to move to either of the pole. C-metaphase, possibly reversible, may occur due to interrupted microtubules and could be induced aneuploidies due to the effect of *At*-ext (21). Polyploidies could occur due to a lack of phragmoplast (33). Binuclear cells occur as a result of cytokinesis inhibition (40). As seen in Table 3, anaphase-telophase (except at 12, 24, and 96 h) and other anomalies (except at 96 h) in MMS were lower than in other applications of *At*-ext.

Results of the Ames test are shown in Table 4. In these experiments, first the cytotoxic doses of *At*-ext were determined. As seen in Table 4, spontaneous revertants were within normal values in all strains examined. Doses of 1000, 100, and 10 µg/plate without S9 mix in TA98; 10, 1, and 0.1 µg/plate doses without S9 mix in TA100; and all doses of *At*-ext with S9 mix in TA100 slightly decreased spontaneous reversion. On the other hand, plates containing positive control mutagens displayed very significant increases in the spontaneous mutation rate in 2 strains tested. Most of the results, whether increasing or decreasing relative to the negative control group, were not statistically significant at $P < 0.05$ (Duncan's test) in examined strains, except 100 µg/plate doses of *At*-ext in TA100 with S9 mix. Ames test results showed that all applications of *At*-ext were not mutagenic for *S. typhimurium* TA98 and TA100, with or without S9 mix. In establishing the dose-response relations, 5 different concentrations of *At*-ext were tested. We observed no induced revertants in either the presence or absence of S9 mix in 2 strains tested.

A. millefolium tea was reported to have a weak genotoxic effect, and this could be due to the presence of flavonoids (41). A significant increase in mitotic recombinants, due to either the initiation of mitotic non-disjunction or cross-over, was reported

Table 4. Mutagenicity of *At*-ext towards *S. typhimurium* TA98 and TA100 strain, with or without S9 mix.

	Amount (µg/plate)	No of His^+ revertant/plate mean ± SD*			
		TA98		TA100	
		–S9 mix	+ S9 mix	–S9 mix	+ S9 mix
At-ext	1000	26 ± 2a	30 ± 3.16a	160 ± 2.34c	175.60 ± 10.50ac
	100	26.20 ± 1.48a	32 ± 2.73a	121.8 ± 2.86ac	129.80 ± 4.91c
	10	29.20 ± 1.92a	28.20 ± 2.48a	117.2 ± 4.43a	145.60 ± 4.82ac
	1	31 ± 3.16a	31.40 ± 2.50a	113.2 ± 3.42a	158 ± 5.14ac
	0.1	31.80 ± 2.58a	24.60 ± 1.14a	110.6 ± 7.89a	173.60 ± 3.43ac
Control		29.20 ± 2.23a	22 ± 1.22a	121.20 ± 2.38ac	192.40 ± 6.26a
SA	10			1726.20 ± 77.44b	
2AA	5				2496.20 ± 99.72b
2AF	200		3144.40 ± 80.92b		
NPD	200	3195.82 ± 109.91b			

*: different letters differ significantly at the level of 0.05; SD: standard deviation; SA: sodium azide; 2AA: 2-aminoanthracene; 2AF: 2-aminofluorene; NPD: 4-nitro-o-phenylenediamine.

by De Sant'anna et al. (42). The effect of *Achillea* on spermatogenesis has revealed contradictory results. Montanari et al. (43) showed an antispermatogenic effect that could be due to cytotoxic chemicals. Dalsenter et al. (44) reported that although body weight did not differ among study groups there was a significant increase in the number of abnormal sperm in the *A. millefolium* aqueous crude extract group. Takzare et al. (45) suggested that extract of *A. millefolium* L. showed a temporary anti-fertile effect in adult male Wistar rats. The anticancer agent apresin, isolated from flowers of the aerial parts of *A. clavenna,* has shown cytotoxic activity (46). Ghantous et al. (47) showed that certain seco-tanapartholides isolated from *A. falcate* decreased keratinocyte cell viability. Teixeira et al. (48) found no statistically significant alterations with *A. millefolium* leaf extracts for acute mutagenicity. An antimitotic effect was only observed with the higher concentration of *A. cepa*; however, this effect was reversible. *A. millefolium* infusion (3.5×10^{-4} g/L) was non-clastogenic, per se; however, it was able to affect the clastogenic action of MMC and Ara-C on DNA break induction (49).

As mentioned above, the results obtained from *Achillea* are contradictory due to location, plant part (e.g., root, stem, fruit, flower, leaf), preparation method (e.g., tea, decoction, infusion), state (e.g., natural or dehydrated), concentration, and duration of treatment. In conclusion, our results indicate that *At*-ext was cytotoxic and genotoxic by *Allium* test; however, it was not mutagenic by Ames test. Although some *Achillea* species have been used in folk remedies for various purposes, they can also cause serious problems and damage when used at high dosages. Thus, care should be taken when using them. Finally, some additional studies should be carried out to determine *A. teretifolia*'s biologically active ingredients or compounds, and the cytotoxic, genotoxic, and/or mutagenic effects of these compounds should be studied.

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