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POTENTIAL CYTOTOXICITY OF *ACHILLEA WILHELMSII* C. KOCH COLLECTED FROM DIFFERENT ALTITUDE ON *DONALIELLA SALINA* AND *ALLIUM CEPA* ROOT TIP CELLS

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Abstract

To contribute to the evaluation of potential cytotoxicity of medicinal plant *Achillea wilhelmsii* C. Koch, aerial parts of the plant were collected from different altitudes of Boyer-Ahmad and Dena districts in Kohgiloyeh and Boyer-Ahmad Province in Iran. Then, the effects of aqueous extracts of aerial parts on *Allium cepa* root tip meristematic cells and on the viability of unicellular, naked green algae *Dunaliella salina* were investigated. Number of cells in prophase, metaphase, anaphase and telophase decreased in the presence of extracts relative to the control. All the extracts reduced mitotic index in a dose–dependent manner. In the Boyer–Ahmad district, extracts of the plants growing in lower altitudes exhibited more cytotoxicity which is evident by the higher reduction in mitotic indices. This relationship was not observed in Dena district. Maximum root length and mean of all individual root length in each treatment reduced with the increase in extracts concentrations. In testing cell viability, progressive reduction in *D. salina* cell number occurred with rise in the extracts concentrations. It is concluded that despite the profound therapeutic advantages of the *A.wilhelmsii*, the cytotoxicity and the genotoxicity of this medicinal plant need to sufficiently be investigated and their possible adverse effects on human health resulting from long–term use of such plants must be evaluated.

Key words: *Achillea wilhelmsii*, altitude, mitotic index, viability, root tipmeristematic cells.

Introduction

Achillea wilhelmsii C. Koch is a perennial medicinal herb with relatively wide distribution in different parts of Iran. It contains various secondary plant products including flavonoids and sesquiterpene lactones (1). Several pharmacological properties of this plant such as antimicrobial, antinflammation, antitumor, antispasmodic and hepato

protective activity have been reported(2,6).Despite the profound therapeutic advantages of medicinal plants, many plants used in traditional and folk medicine are potentially cytotoxic and genotoxic (3,4,5).This raises questions about the hazards resulting from the long–term useand exposre to such medicinal plants (8). Using both MCF-7 cell line and root meristems of *Allium cepa*, it was shown that *Euphorbia hirta* exerted significant cytotoxic and genotoxic effects (7,9). *Arctium lappa* which has been used in folk medicine for its anti-inflammatory, antioxidant and anti HIV activites, showed cytotoxicity and genotoxicity on the root meristem of *Allium cepa*. Inhibition of cell division and, as a result, reduction in mitotic index were observed in *A. cepa* root meristemtic cells(8).Several others herbal extracts have been reported to inhibit mitosis (10,11,12).

The root meristematic cells of *A. cepa* has proven to be very good material to study cytotoxicity and genotoxicity of various substance and has been repeatedly suggested as a standard test system (15).It is an established plant bioassay validated by the International Programme on Chemical Safety (12) and the United Nations Environment Programme (14) as an efficient and standard test for monitoring the toxicity of environmental chemicals. The test is easy to handle, has low cost and the most important is its good correlation with other test systems including mammalian test systems (16).

Due to limited reports on the cytotoxicity of *Achillea wilhelmsii* C. Koch, in the present study, the effects of aqueous extracts of *A.wilhelmsii* aerial parts collected from seven locations of Kohgiloyeh and Boyer-Ahmad province on the root growth and mitotic index of *A. cepa* root meristematic cells and cell viability of *Dunaliella salina* were investigated.

Materials and Methods

Sample collection and preparation

At the flowering stage, *A. wilhelmsii* aerial parts were collected during the summer of 2013 from four locations in Dena district (Padena 2448 m, Amirabad 1890 m, Bahrambigy 1800 m and Kare 1739 m) and three locations in Boyer–Ahmad district (Galal 2373 m, Thlion 1793 m and chin 1485 m above sea level) of Kohgiloyeh and Boyer-Ahmad province and identified by Dr. Khosravi at the herbarium of biology department, Shiraz University. The samples were rinsed with distilled water and air–dried in the dark at room temperature.

Ten grams of each finely ground sample was extracted with100 ml distilled water in a foil rapped Erlenmayer flask with continuous shaking for 24 hrs at room temperature. The extracts were filtered and centrifuged at 4000 rpm for 15 min and the supernatants were used for root length, mitotic index and cell viability determinations.

Allium cepatest

Onion bulbs of uniform size (about 20 gr) were selected and the outer scales were removed and the apices of the root primordial were exposed. Onions were placed on top of glass vessels containing tap water with the bottom plates containing root primordial submerged in the water. The glass vessels were kept in the dark at room temperature. Onions with root length of about 1.5 cm, were transferred to solutions containing 25, 50 and 75 mg ml^{-1} of original extracts and kept in the dark for 48 hrs. For mitotic index determination, root tips with 0.5 cm in length were collected and fixed in 96% ethanol: glacial acetic acid 3:1(v/v) for five hrs. Then, the root tips were transferred to 33% acetic acid solution for 30 min. After fixation, the tips were kept to 1.0 N HCl solution for 5 min. The tubes containing the root tips were heated for 10 min in a water bath set at 60^0C. After washing the tips with distilled water, they were stained with Feulgenreagent as described by (17) and slides for microscopical studies were prepared according to (18,19). Each experiment had three replication and 700 cells were scored for each treatment. Mitotic index was calculated as the ratio between the number of dividing cells to the total number of scored cells and expressed as percent mitotic index.

As macroscopic parameters (20), for each treatment, maximum root length and the mean value of all the roots lengths in a bulb are reported. Cell viability was determined using unicellular, naked green algae *Dunaliella salina* strain MSV–3.This strain was previously isolated and identified bused on morphology and r DNA ITS Sequence (21). For each treatment, in a glass vessel, 1.0 ml of plant extract was added to 3 ml solution culture containing 6×10^5 cell ml^{-1}.The glass vessels were kept under continuous light provided by white fluorescence lamps at an intensity of 3500 lux and 25 ^{0}C for 24 hrs. Then, to 1.0 ml of algal culture was added 10 µl Lugol solution which immobilized algal cells. The cells were counted using a hemocytometer and cell viability was calculated by dividing number of alive cells to total cell number and expressed as percent viable cells. Each treatment had three replicates and 2100 cells were counted for each replicate.

Statistical analysis

Data are expressed as mean of three replicates$\pm$ standard error. Means were compared using Duncan's test of significance at $p\leq 0.05$. SPSS version 16 was used for data analysis.

Results and Discussion

As shown in Table 1, the mitotic index in *A. cepa* meristematic root tips, treated for 48 hrs with different concentration of *Achillea wilhelmsii*aerial parts extracts, decreased significantly with increase in extract

concentration. Highest decrease was recorded in plants growing in Chin. As with total reducing capacity and free radical scavenging potential reported earlier (22), in Beyer–Ahmad district decrease in altitude was accompanied by higher cytotoxicity of the extracts which is evident by lower mitotic indices. Such a relationship between the altitude and the mitotic index was not observed in Dena district. Concomitant with decrease in mitotic index, root length of *A.cepa* reduced with increase in the concentration of *A.wilhemsii* extracts (Table2).When the unicellular green algae *D. salina* was used as a model system to determine the cytotoxicity of the *A.wilhelmsii* extracts, it was found that all the extracts decreased cell viability (Table3). Higher extracts concentrations caused more reduction in cell viability. Plants collected from Kare and Chin regions, showed the highest cytotoxicity compared to plants sampled from other regions (Table3). At 100 mg ml^{-1}, cell viability reduced to 5.1 and 3.9 percent in Kare and Chin, respectively. Extract from samples collected at Galal region had the lowest cytotoxicity. In general, no relationship was observed between the altitude and cytotoxicity of the extracts. It has been stated that crude therapeutic products from medicinal plants are less toxic compared to their synthetic counterparts (23). One suggested reason is the presence of many medicinal compounds in an extract, just as they are found in their natural source and so have less risk of side effects. These compounds may act synergistically with the active compounds as well as antagonistically to reduce the side effects(25).On the other hand, it has been shown that some herbal medicines can potentially be toxic to human health and many plants used in traditional medicine are potentially cytotoxic and genotoxic (24). Using root tip cells of *A. sativa* as test system, it was shown that high concentration of tobacco leaf extract are mitodepressant(26). While carvacrol slightly increases apoptotic cell death, thyme oil has relatively high cytotoxicity which increases both apoptotic and necrotic cell death (27). Cytotoxic and genotoxic effects of leaves aqueous extracts of medicinal plants *Lantana camara* and *Lippia alba* on *Lactoca sativa* root tip meristematic cells was reported by Sousa et at. (28). Reduced mitotic index, seed germination and root development and increased chromosomal aberrations was observed after 72 hrs exposure to the extracts. Cytotoxic compounds can result in decreased cell division and growth and also cause loss of membrane integrity with subsequent decrease in cell viability. Despite the profound therapeutic advantages of the medicinal plants and health benefits from their continued utilization in folk medicine, the cytotoxic and genotoxic hazards resulting from long – term use of medicinal plants need to sufficiently be investigated.

Table-1: Effects of different concentration of *A. wilhelmsii* on prophase (P), metaphase (M), anaphase (A), telophase (T) and mitotic index (mean±SE, %) of the *Alliumcepa* root meristematic cells. Altitudes of locations are given in meters above sea level.

District	Location	*A.wilhelmsii* extract (mg ml^{-1})	P	M	A	T	Mitotic index (%)
Dena	Padena (2448)	10	261	1	1	0	37.26±0.66
		25	194	0	0	0	27.3±0.33
		50	161	0	0	1	23±0.0
		75	112	0	0	0	16±0.11
	Amirabad (1890)	10	195	5	1	2	29±1.15
		25	164	0	0	14	25.33±0.33
		50	122	0	0	0	17±0.57
		75	98	0	0	0	14±0.75
	Bahrambigy (1800)	10	177	1	2	8	27±0.57
		25	159	5	3	15	26±0.0
		50	160	2	0	2	23.4±0.23
		75	111	2	1	0	15.83±0.44
	Kare (1739)	10	254	4	4	10	37.33±0.33
		25	218	6	5	3	33.06±0.066
		50	164	3	4	9	25.33±0.33
		75	120	2	5	0	18.2±0.2
Boyer-Ahmad	Galal (2373)	10	259	3	3	1	38±0.57
		25	166	2	1	0	23.66±0.33
		50	147	3	2	1	21.33±0.33
		75	115	0	2	2	16.83±0.44
	Thlion (1793)	10	219	0	0	0	31.33±0.33
		25	174	1	1	7	26.33±0.33
		50	137	0	0	1	19.33±0.33
		75	95	0	0	1	13.33±0.33
	Chin (1485)	10	188	4	3	4	28±0
		25	148	0	0	2	21.33±0.33
		50	108	2	2	1	16.13±0.13
		75	75	2	1	1	11.06±0.58
		control	303	16	12	4	47±1.15

Table-2: Effects of aqueous extracts of *A. wilhelmsii* aerial parts collected from different locations on the maximum root length (percent relative to control) and mean length of all individual roots in each treatment.

District	Location	*A.wilhelmsii* extract (mg ml^{-1})	Maximum root length (%)	Mean of all roots (%)
	Padena	25	57.4±0.28	47.8±0.057
		50	42.5±0.57	43.4±0.115
		75	46.8±0.115	34.7±0.11
		100	48.9±0.17	30.4±0.057
	Amirabad	25	47.4±0.28	35±0.047
		50	42.3±0.115	28.3±0.115
		75	33.3±0.23	21.6±0.11

Dena		100	17.9±0.17	6.6±0.057
	Bahram bigy	25	29.1±0.057	16.6±0.28
		50	55.5±0.28	25±0.173
		75	20.8±00.17	10±0.115
		100	23.6±0.24	8.3±0.057
	Kare	25	55.1±0.057	50±0.17
		50	32.7±0.05	30±0.088
		75	51.7±0.402	23.3±0.115
		100	31.03±0.405	16.6±0.115
Boyer-Ahmad	Galal	25	50.7±0.054	30±0.115
		50	45.07±0.112	20±0.115
		75	42.25±0.28	13.3±0.112
		100	28.1±0.57	23.3±0.11
	Thlion	25	49.09±0.11	37.03±0.11
		50	32.7±0.10	40.17±0.057
		75	38.1±0.051	37.03±0.12
		100	36.2±0.05	18.5±0.115
	Chin	25	45.2±0.23	33.3±0.56
		50	32.07±0.114	41.6±0.057
		75	32.07±0.17	29.1±0.057
		100	28.3±0.169	12.5±0.057

Table-3: Cytotoxicity of aqueous extracts of *A. wilhelmsii*aerial parts sampled from locations with different altitude on *Dunaliella salina* cell viability. Values are percent living cells relative to control.

location	Extract concentration (mg ml^{-1})				
	10	25	50	75	100
Padena	89.7 ± 4.17^{ab}	71.7 ± 6.7^{b}	49.5 ± 9.5^{c}	26.2 ± 7.2^{d}	21.29 ± 9.4^{d}
Amirabad	82.6 ± 1.13^{ab}	71.13 ± 1.98^{b}	67.7 ± 10.7^{b}	41.2 ± 7.7^{c}	18.4 ± 5.2^{d}
Bahrambegy	77.3 ± 2.19^{b}	68.2 ± 3.2^{bc}	52.2 ± 0.47^{cd}	35.6 ± 5.9^{de}	27.7 ± 11.5^{e}
Kare	67.8 ± 8.99^{b}	68.6 ± 5.06^{b}	20.1 ± 1.66^{c}	15.3 ± 5.60^{c}	5.1 ± 2.55^{c}
Galal	93.1 ± 2.1^{b}	88.8 ± 2.38^{b}	50 ± 2.88^{c}	44.4 ± 1.6^{c}	34.03 ± 2.53^{d}
Thlion	84.3 ± 2.6^{b}	62.03 ± 3.5^{c}	41.02 ± 6.63^{d}	28.6 ± 7.3^{d}	3.9 ± 3.2^{e}
Chin	87.2 ± 2.53^{ab}	83.2 ± 7.37^{ab}	73.6 ± 14.7^{bc}	53 ± 1.15^{c}	19.8 ± 0.44^{d}

Each value is mean $\pm$ SE. Different letters in each row are significantly different ($P\leq 0.05$) from each other. The letter "a" shows no significant different with the control.

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