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Antiradical and cytotoxic activity of *Origanum* species extracts

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ABSTRACT

The aim of this study is to determine the antiradical activity (DPPH decolouration test) and cytotoxicity (*Artemia salina* lethality test) of aqueous extracts (AE) and essential oils (EO) of *Origanum* sp.
Origanum x *applii* EO was the most active and exhibits only 12% of the antioxidant capacity of ascorbic acid in equal initial concentrations (100 ppm). Under these conditions, ascorbic acid exhibited decolouration of 97%, and 96% for 50 ppm. AE of *O.* x *majoricum*, *O. compactum* and *O.* x *applii* showed decolouration of 82%, 90% and 89% respectively for 30ppm, when tested immediately after preparation. Kept at 4 ° C, and after 24 hours, a decrease of 90% in the antioxidant capacity of ascorbic acid and only 2% in the aqueous extracts was observed. EO had low LD50 values (10-29 ppm) indicating cytotoxic activity. AE of *O.* x *majoricum* showed LD50 of 5546ppm and *O.* x *applii* yielded LD50 values comparable to water (negative control).
EO from *Origanum* sp show low antioxidant activity but high toxicity against *Artemia salina*. Therefore EO should be tested for other biological activities.
On the other hand AE show low toxicity and high antioxidant capacity which encourages further studies to detect compounds responsible for antiradical activity.
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Keywords: *Origanum* sp-Antiradical activity-Essential oils-Aqueous extracts

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Introduction

The use of herbs as medicine is as old as humanity. Its use is widespread throughout the world (Principe, 1989). Medicinal plants are source of therapy for most population living in the Third World. A number of healing properties are attributed to them.

Besides the traditional use of *Origanum* species as a spicy additive for food, the genus has several applications in folk medicine as sedative, diuretic, degasifier, sweater and antiseptic, and also in the treatment of gastrointestinal diseases and constipation (Baytop, 1999)

Some plant compounds can reduce the deleterious effects of reactive oxygen species (ROS) on a number of biological and pathological processes. Scavenging of ROS by plant compounds may be the basis of the purported human health benefits of plants (Sawa *et al.*, 1999). Beverages such as herbal infusions and teas that do not have any particular nutritional value also constitute an important source of antioxidants (Warren, 1999). Then, herbal infusions and teas could be taken as a good complement of the antioxidants intake in the human diet. Thereby, the antioxidant capacity of herbal infusions and teas have been studied in different in vitro systems such as DPPH antioxidant assay (Schmeda-Hirschmann *et al.*, 2003)

Mortality *in vivo* of a single organism in the zoological scale can be used as a suitable monitor for the screening, fractionation and detection of biologically active natural products (McLaughlin, 1997). The BST (Brine Shrimp test or *Artemia* test) method was developed to test cytotoxicity. *Artemia salina* nauplii are often used as the organism to perform this test.

García Ocón *et al.* (2009) using *A. salina* and commercial amikacin found a correlation between the concentration of a toxic compound versus its lethality, while Barbosa (2008) found that the compound 2-[hydroxyl (4-bromophenyl) methyl] acrylonitrile is both active against leishmania and highly toxic to *A. salina*. This indicates that these organisms can be used in a preliminary test of biological activities.

This assay was used successfully in many species and by various authors (McLaughlin *et al.* 1995 and 1997, García Ocón *et al.* 2009, Viturro *et al.*, 2008).

The aim of this study is to determine the antiradical activity of aqueous extracts (AE) and essential oils (EO) of *Origanum* sp grown in the Quebrada de Humahuaca, Jujuy, Argentina, and at the same time evaluate the cytotoxicity of these extracts.

Experimental

Plant material:

Aerial parts of three oregano species grown in Tilcara (2460 m), Jujuy, Argentina, were collected and identified as: *Origanum* x *majoricum*, *Origanum compactum* and *Origanum* x *applii*.

The aerial parts were dried and stored for analysis.

Essential oils

Essential oils were obtained by hydrodistillation (using a Clevenger type trap) of leaves, stems and flowers from *O. x majoricum*, leaves and flowers from *O. compactum* and dried leaves and stems from *O.* x *applii* at the beginning of flowering.

Aqueous extracts

Aqueous extracts were prepared according to traditional use (1%) adding boiling water to dried, powdered vegetal and boiling during one minute. Percentage of soluble solids was determined with an OHAUS MB45 moisture analyzer.

Cytotoxicity

Cytotoxicity was determined using the Brine shrimp lethality test (BST) as described by Meyer (1982).

Essential oils were tested (Viturro *et al.*, 2008) with initial concentrations of 1, 10 and 100 ppm (µL/L).

Solutions of 1, 10 and 100 ppm (µg soluble solid/mL) of aqueous extracts were prepared for the assay.

All assays were conducted in triplicate.

Antioxidant activity

DPPH decolouration test was performed after the technique described by Joyeux (1995). DPPH (1,1-diphenyl-2-picrylhydrazyl) methanol solutions that absorb at 517 nm (violet) were used. Tests were conducted in triplicate and ascorbic acid was used as positive control.

Initial concentrations were 100, 1000 and 10000 ppm (µL/L) for essential oils and 0.3, 3, 30 y 300 ppm (µg soluble solid/mL) for aqueous extracts.

Results and Discussion:

Percentage decolouration of DPPH produced by EO and AE is shown in Table 1.

Origanum x *applii* EO was the most active and exhibits only 12% of the antioxidant capacity of ascorbic acid in equal initial concentrations (100 ppm). Under these conditions, ascorbic acid exhibited decolouration of 97% and 96% for 50

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ppm (µg/mL). AE of *O.* x *majoricum*, *O. compactum* and *O.* x *applii* showed decolouration of 82%, 90% and 89% respectively for 30ppm (µg soluble solid/mL), when tested immediately after preparation.

Kept at 4 ° C, and after 24 hours, a decrease of 90% in the antioxidant capacity of ascorbic acid and only 2% in the aqueous extracts was observed.

The results regarding the cytotoxic activity are expressed as LD50 (concentration required to achieve 50% mortality of nauplii).

EO had low LD50 values (10-29 ppm) indicating cytotoxic activity.

AE of *O.* x *majoricum* showed a LD50 of 5546 ppm while those of *O.* x *applii* yielded LD50 values comparable to those of water (used as negative control)

Conclusions:

Essential oils from *Origanum* sp show low antioxidant activity but high toxicity against *Artemia salina*. Therefore EO should be tested for other biological activities.

On the other hand *Origanum* aqueous extracts show low toxicity and high antioxidant capacity which encourages further studies to detect compounds responsible for antiradical activity.

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Table 1. Antioxidant activity of *Origanum* sp essential oils and aqueous extracts using DPPH method

	Sample	Initial concentration (1)	Final concentration (1)	Mean decoloration percentage	Standard deviation %
	Ascorbic acid	10	3	90	0.4
		50	17	96	0.3
		100	33	97	0.7
Essential oils	*Origanum x majoricum*	100	33	2	1.6
		1000	333	22	0.6
		10000	3333	70	1.0
	Origanum compactum	100	33	6	2.8
		1000	333	31	7.4
		10000	3333	73	0.3
	Origanum x *applii*	100	33	12	1.7
		1000	333	51	1.7
		10000	3333	76	1.8
Aqueous extracts	*Origanum x majoricum*	0.3	0.1	2	28
		3	1	14	1.2
		30	10	82	7.7
		300	100	90	0.2
	Origanum compactum	0.3	0.1	3	1.8
		3	1	14	0.8
		30	10	90	0.3
		300	100	89	1.5
	Origanum x *applii*	0.3	0.1	2	1.4
		3	1	13	0.4
		30	10	89	0.2
		300	100	91	0.2

(1) In μL/L for essential oils and μg soluble solids/mL for aqueous extracts