Biological activity of ethanol extract from leaves of *Rosmarinus eriocalyx*.

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Introduction

Aging or senescence is a complex and inevitable process, which is not only attributed to individual genetic variation but also to external factors such as environmental conditions, nutrition, alcohol, and diseases [1]. The most widely accepted theory, that have been proposed to explain aging, is the free radical theory [2]. Aging and related diseases result from accumulated oxidative damage to cell constituents and tissues caused by excessive exposure to free radicals. Antioxidants, which mediate the imbalance between intracellular antioxidant defenses and oxidative damage by reducing the reactive oxygen species (ROS) levels, are believe to be able to reduce stress-induced premature senescence or slow down replicative senescence [3].

Rosmarinus eriocalyx (Jord. & Fourr.) is an aromatic evergreen bush belonging to Lamiaceae family and endemic to Algeria, Morocco and Spain, where it is used as a condiment to flavor soup and meat and as a traditional remedy [4]. The plant volatile fraction is characterized by the monoterpene ketone camphor, whereas its ethanolic extracts are rich sources of phenolic acids and diterpenes such as rosmarinic acid, carnosic acid and carnosol that are the main responsible for the noteworthy antioxidant activity [5]. In this setting, we aimed to evaluate *R. eriocalyx* biological activity in order to propose the plant as an anti-aging agent.

Fig. 1. *Rosmarinus eriocalyx*

Table 1. Cytotoxic activity of *Rosmarinus eriocalyx* aqueous and ethanolic extracts.

	Tumor cell line (IC_{50} µg/ml)[a]		
	A375[b]	MDA-MB 231[c]	T98G[d]
Ethanolic extracts			
Stems	49.75	83.22	> 100
95% C.I.[e]	40.43 - 61.23	76.20 - 90.89	
Leaves	17.80	30.66	53.94
95% C.I.	14.54 - 21.81	27.82 - 33.80	49.73 - 58.51
Flowers	42.66	61.34	85.39
95% C.I.	37.34 - 48.73	51.32 - 73.32	68.77 - 106.0
Aqueous extracts			
Stems	> 200	> 200	> 200
95% C.I.			
Leaves	> 200	> 200	> 200
95% C.I.			
Flowers	> 200	> 200	> 200
95% C.I.			
Positive control			
Cisplatin	0.40	2.29	2.22
95% C.I.	0.33 - 0.46	2.04 - 2.78	2.02 - 2.45

[a] IC50 = The concentration of compound that affords a 50% reduction in cell growth (after 72 h of incubation). [b] Human malignant melanoma cell line. [c] Human breast adenocarcinoma cell line. [d] Human colon carcinoma cell line. [e] Confidence interval.

Experimental

Inflorescences, stems and leaves of *R. eriocalyx* were collected in Djbel Boutaleb, province of Setif (North-East Algeria, 900–1500 m above sea level, N 35°43'52''; E 5°11'13'') in March 2015. Dried *R. eriocalyx* stems, leaves and flowers were reduced into a powder using a blender MFC DCFH 48 IKA-WERK (D-Staufen). Five grams of each part were weighed and extracted with 50 mL of two different solvents, i.e. water and ethanol. After 24 h the extract was dried with rotavapor, freeze-dried and stored in freezer. Cytotoxic activity of extracts was performed using MTT assay on the following human tumor and non-tumor cell lines: MDA-MB 231 (human breast adenocarcinoma cells), T98G (human glioblastoma multiforme cells), A375 (human malignant melanoma cells), and HuDe (human dermal fibroblast cells). MTT assay was carried out as described previously [4,5]. Cells were incubated with increasing concentrations of aqueous and ethanolic extracts (from 0.78 to 200 µg/mL) for 72 h. Cytotoxicity was expressed as the concentration of compound inhibiting cell growth by 50% (IC_{50}). The cellular antioxidant activity was measured using a modified method from Wolfe et al. [7]. The SA-β-gal activity was determined as described by Kurz et al. [8].

Table 2. Cytotoxic activity of aqueous and ethanolic extracts of *R. eriocalyx* leaves on HuDe human fibroblast cell line.

Rosmarinus eriocalyx	(IC_{50} µg/ml)[a] HuDe[b]
Ethanolic extract	33.98
95% C.I.[c]	30.59 – 37.82
Aqueous extract	> 200
95% C.I.	
Rosmarinic acid	16.35
95% C.I.	11.30 – 23.66

[a] IC50 = The concentration of compound that affords a 50% reduction in cell growth (after 72 h of incubation). [b] Human fibroblast cell line. [c] Confidence interval.

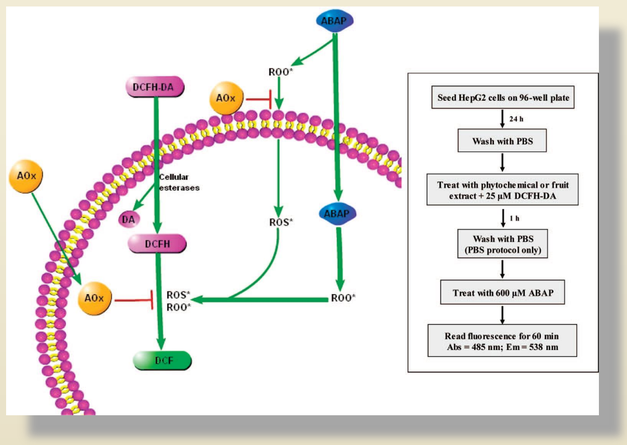

Fig. 2. Method and proposed principle of the cellular antioxidant activity (CAA) assay [7].

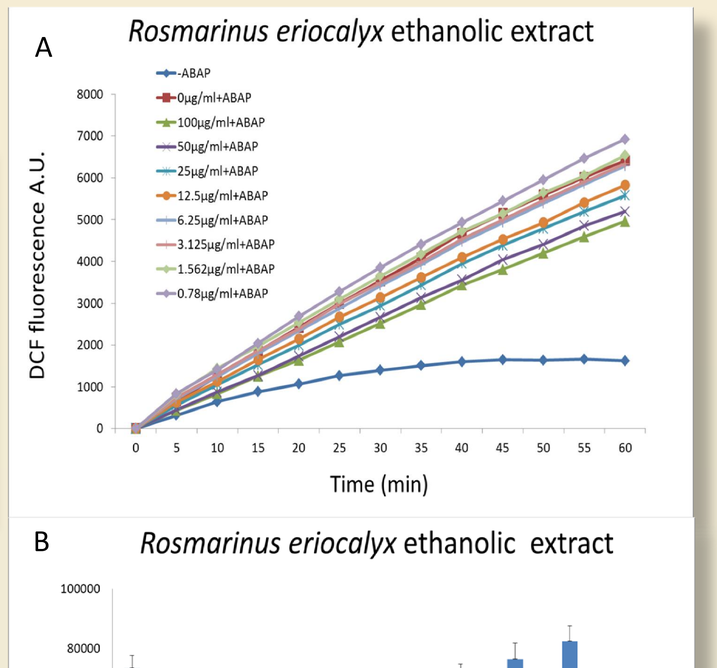

Fig. 3. Peroxyl radical-induced oxidation of DCFH-DA to DCF in Hude cells and inhibition of oxidation by *R. eriocalyx* ethanolic leaf extract over time (A). Dose-response curves for inhibition of peroxyl radical-induced DCFH oxidation by *R eriocalyx* ethanolic leaf extract (B)2',7'-dichlorofluorescin diacetate (DCFH-DA) 2.2'-azobis -2-amidinopropane (ABAP).

Results

Starting from the data obtained in previous work, where aqueous and ethanol extracts of *R. eriocalyx* were characterized in phenolic composition and antioxidant activity, we aimed to evaluate *R. eriocalyx* biological activity in order to propose the plant as an anti-aging agent. For this purpose, we determined the cytotoxic activity of polar extracts obtained from leaves, flowers, and stems of *R. eriocalyx* on human tumor cell lines (A375, MDA-MB 231, and T98G) by MTT assay [6]. The IC_{50} values of *R. eriocalyx* extracts were reported in Table 1. The data showed that the ethanolic extracts resulted active against all cell lines tested. A375 human melanoma cells were the most sensitive to leaf ethanolic extract (IC_{50} value of 17.8 µg/ml). On the other hand, aqueous extracts showed no activity, at least in the range of concentrations tested (0.78 – 200 µg/ml). As reported [5], the total phenolic content values in *R. eriocalyx* ethanolic and aqueous extracts showed slight differences, instead free radical scavenging activity was stronger for ethanolic extracts than aqueous ones. On this basis, we selected the ethanolic extract to determine the cytotoxicity and the antioxidant activity on human dermal fibroblast (HuDe) by measuring its ability to prevent oxidation in cells using a ROS fluorescent probe (DCFH-DA) (Fig. 2) [7]. In Table 2 the IC_{50} value of leaf ethanolic extract was reported. In Fig. 3, the results showed a remarkable activity in preventing oxidation of cells induced by 2.2'-azobis -2-amidinopropane (ABAP) in dose-dependent manner. Afterwards, we tested the same extract on the H_2O_2-induced premature senescence in human fibroblast cells where β-galactosidase (SA-β-gal) activity was used to measure cellular senescence (Fig. 4) [8]. The addition of ethanoic extract during the recovery period to H_2O_2 -treated cells resulted in a significant reduction in the number of SA-β-Gal positive cells.

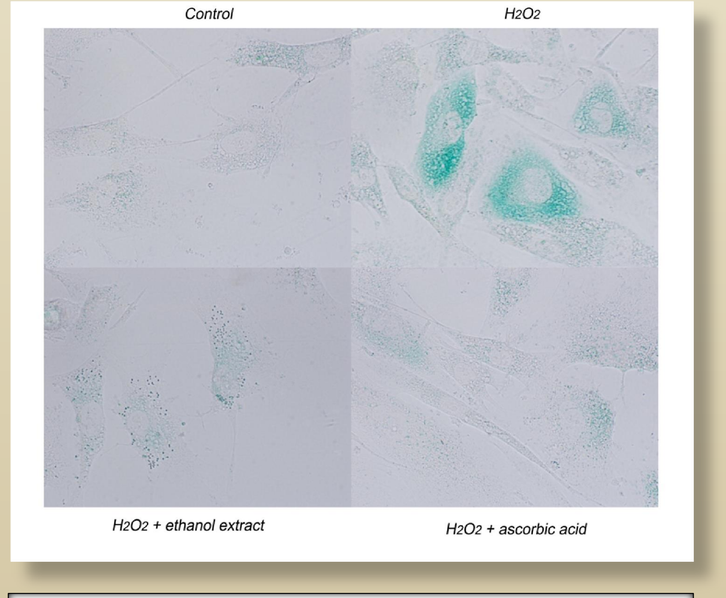

Fig. 4. Recovery effect of *R. eriocalyx* leaf extract on H_2O_2 treated HuDe cells. Cells were treated for 48 h with 25 µg/ml *R. eriocalyx* ethanolic extract or 100 µM ascorbic acid after 2 h incubation with 100 µM H_2O_2 as exogenus stress. SA-β-Gal positive cell staining. Bright field microscopy.

Conclusions

Our results demonstrate that *R. eriocalyx* ethanolic extract possessed antiproliferative activity against human tumor cell lines and antioxidant activity on cell system as well as *in vitro* system. H_2O_2 stress-induced premature senescence can be significantly suppressed by *R. eriocalyx* leaf extract. Preliminary data suggest the therapeutic potential of *R. eriocalyx* extract as an anti-aging agent.

References

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