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Antioxidant activity in heterogeneous and homogeneous system of the resinous exudates from *Heliotropium stenophylum* and *H. sinuatum* and of 3-O-methylgalangin their main component

[Actividad antioxidante en sistema heterogéneo y homogéneo de los exudados resinosos de *Heliotropium stenophyllum* y *H. sinuatum* y de 3-O-methylgalangina su componente mayoritario]

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Abstract
The antioxidant activity of resinous extracts obtained from *H. stenophylum* and *H. sinuatum* species, was evaluated through ORAC index (Oxygen Radical Absorbance Capacity) in water phase and in presence of Triton X-100 micelles, using as test molecules to pyrogallol red (PGR) and evaluating their reduction by the action of peroxyl radicals obtained from thermolysis of AAPH. The results show that these extracts protect to PGR of the action of the radicals. This protection is reduced drastically in the presence of Triton X-100 micelles. The same effect was observed with the main flavonoid of these extracts (3-O-methylgalangin). These results show the importance of the media of reaction of pure compounds and/or extracts at the time of to take into account their use as antioxidants.

Keywords: antioxidant, flavonoid, ORAC-PGR, resinous exudate, micelles.

Resumen
La actividad antioxidante de exudados resinosos obtenidos desde las especies *H. stenophylum* y *H. sinuatum*, fue evaluada a través del ensayo ORAC (Oxygen Radical Absorbance Capacity) en fase acuosa y en presencia de micelas de Triton X-100, usando como molécula prueba a pirogalol rojo (PGR) y evaluando su reducción frente a la acción de radicales peróxidos obtenidos desde la termólisis de AAPH. Los resultados muestran que estos extractos protegen al PGR de la acción de los radicales. Esta protección es reducida drásticamente en presencia de micelas de Tritón X-100. El mismo efecto fue observado con el flavonoide mayoritario de estos extractos (3-O-metilgalangina). Estos resultados muestran la importancia de considerar el medio de reacción de compuestos puros y/o extractos al momento de tomar en cuenta su uso como antioxidantes.

Palabras Clave: antioxidante, flavonoide, ORAC-PGR, exudados resinosos, micelas.

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INTRODUCTION

Currently, there is an increase in the interest by the use of antioxidant natural obtained from plants used in popular medicine (Ozsoy *et al*., 2008; Di Carlo *et al*., 1999) and food products, such as soy, tea, coffee and red wine, among others (Avello and Suwalsky, 2006).

The *Heliotropium* genus, Heliotropiaceae family, it is characterized by living in arid areas and be exposed to extreme environmental factors. For this, their species produce a resinous exudate that cover both leaves and stem and it is secreted by special glands called trichomes (Johnston, 1928). The resin is composed by terpenoids, lipids, waxed and flavonoids, that act as a protective barrier against aggression such as high temperature, radiation UV, water scarcity, herbivores and pathogens (Dell and Comb, 1978).

Previous studies have shown that the components of this resin, have chemical and biological properties, such as: antimicrobial, antiviral and anti-fungal, increasing the resistance of the plants. (Martínez, 2005; Modak *et al*., 2009a, Modak *et al*., 2009b, Modak *et al*., 2010). Of these properties, the antioxidant activity highlights (Modak *et al*., 2003; Modak *et al*., 2005; Modak *et al*., 2007; Modak *et al*., 2009a; Modak *et al*., 2009b; Modak *et al*., 2010).

In particular, *H. stenophylum* and *H. sinuatum* species produces a resinous exudate composed by flavonoids as main component accompanied of 3 H-Spiro[1-benzofuran-2,1'-cyclohexane] derivatives in smaller amounts (Villarroel and Urzúa 1990; Torres *et al*., 1996).

Previous studies of antioxidant activity of the resinous extracts of these species and of its flavonoids pure, using the discoloration of DPPH method in homogeneous phase, showed their ability to capture the radical free. However, the activity of these extracts and flavonoids resulted to be less to the vitamin E and Trolox activities (Lissi *et al*., 1999). Based on these results, arose the idea of study the effect of the environment in the antioxidant activity of flavonoid and of these mixtures. For this, the evaluation of antioxidant activity using the ORAC-PGR test (Romero *et al*., 2010) was carried out in homogeneous phase and in the presence of Triton X-100 micelles. The results are presented in this paper.

MATERIALS AND METHODS

Plant material

Heliotropium stenophyllum Hook.& Arn. was collected during the flowering season in Los Vilos (IV region, Chile, 31º52`S,71º29`W). *Heliotropuim sinuatum* (Miers) was collected during the flowering season in the north of Vallenar (III region, Chile, 29º57`S,71ºW). Voucher specimens were deposited in the Herbarium of the Faculty of Biological Science of Catholic University of Chile. (ST2560 *Hst*; ST2563 *Hsin*).

Extraction and isolation of resinous exudate and flavonoid 3-O-methylgalangin.

The resinous exudates were extracted by immersion of the fresh plant material in dichloromethane for 60 s at room temperature. The extracts were concentrated to a sticky residue.

The extracts were purified by column chromatography (silica gel) using a hexane-ethylacetate step gradient yielding 3-O-methylgalangin as major component (Torres *et al*.,1996).

Antioxidant activity

Evaluation of antioxidant capacity in phosphate buffer (pH 7.4, 75 mM) and in Triton X-100 (50 mM) solution

To evaluate the consumption of PGR (5uM) caused by the addition of AAPH (10 mM), the absorbance was measured at 540 nm (UV-VIS spectrophotometer Shimadzu UV-160) as a function of time at 37° C. These measurements were realized in the presence of different concentrations of resinous exudates and pure compounds (3-O-methylgalangin and Trolox). All measurements were performed in triplicate and the results were expressed as mean ± standard deviation.

Octanol/water partition

The partition of 3-O-methylgalangin and resinous extracts in octanol/water was evaluated. Aqueous solutions (phosphate buffer 75 mM, pH 7.4) of each system were divided into two portions. A portion was added to decantation funnel and was treated with octanol, shaking by 20 minutes. The aqueous phase was separated and its antioxidant activity was evaluated and compared with the activity of the portion not treated with octanol.

RESULTS AND DISCUSSION

Figure 1 shows the kinetic profiles of the reaction of PGR and peroxyl radicals generated by AAPH thermolysis in absence and in presence of resinous extracts. The presence of the extracts produces a shift to the right of the curve, which is interpreted as a

protection of the PGR against the action of the peroxyl radical. To compare the profiles obtained with both extracts, note that at equal concentration, the *H. stenophyllum* resin has greater antioxidant activity. These results coincide with those obtained using the radical DPPH (Lissi *et al*., 1999).

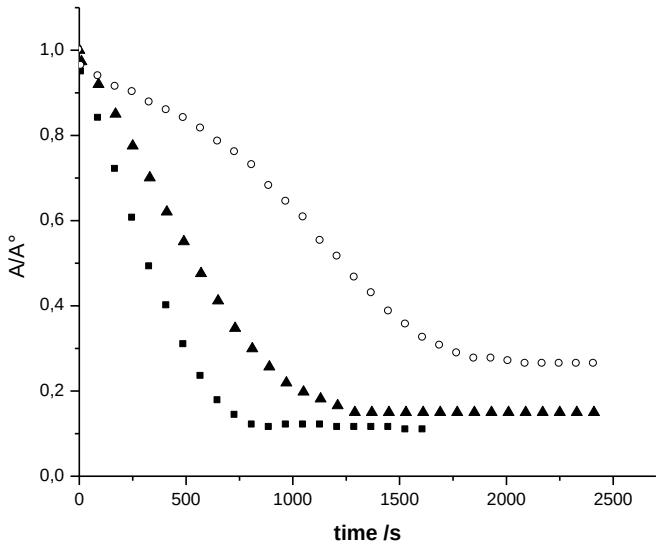

Figure 1
Kinetic profiles of PGR consumption in presence of (▲) *H. sinuatum* 0.05 mg/mL; (o) *H. stenophyllum* 0.05 mg/mL and (■) Control experiment (without extract) in homogeneous media. PGR 5 µM, AAPH 10 mM, 37° C.

Figure 2 shows the bleaching of PGR elicited by peroxyl radicals in absence and in presence of Triton X-100 micelles. These data show that PGR consumption is nearly independent of the surfactant addition. These results agree with those reported by Romero (2010), and indicate that, despite PGR incorporation to the micelles, its rate of consumption is not affected by the surfactant.

In Figure 3, the kinetic profiles of 3-O - methylgalangin in absence and presence of micelles are given. From this figure it can be concluded that the protection provides by this flavonoid, decreases in micellar media. This reduced protection can be explained in terms of flavonoid incorporation into the micelles. This incorporation is favoured for its high hydrophobicity. This is demonstrated by the results obtained through the evaluation of antioxidant activity measured before and after treatment with octanol (Figure 4).

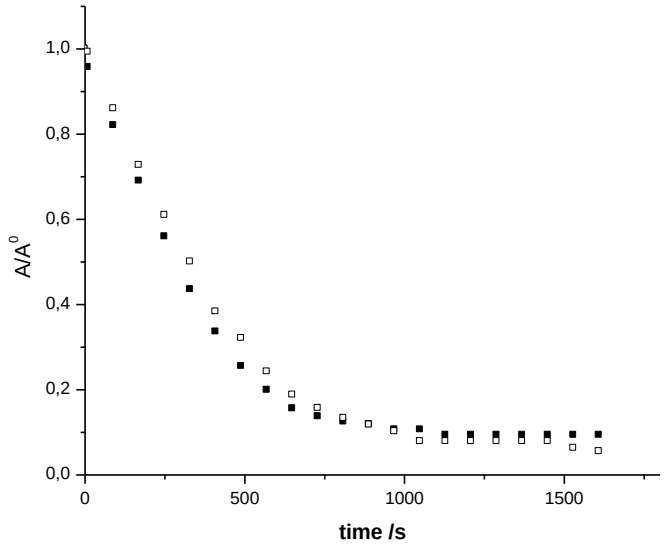

Figure 2
Kinetic profiles of PGR consumption in presence (□) of Triton X-100 micelles (50 mM) and in absence (■) of micelles. PGR 5 µM, AAPH 10 mM, 37° C.

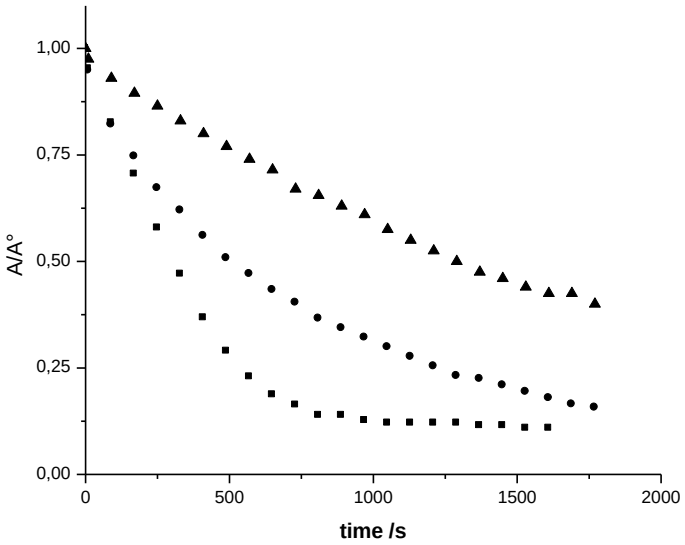

Figure 3
Effect of 3-O-methylgalangin on the consumption of PGR induced by AAPH derived peroxyl radicals in the absence (▲) and presence of Triton X-100 micelles (●) to 1.5 mM and 1.8 mM respectively, and control experiment (■). PGR 5 µM, AAPH 10 mM, a 37° C.

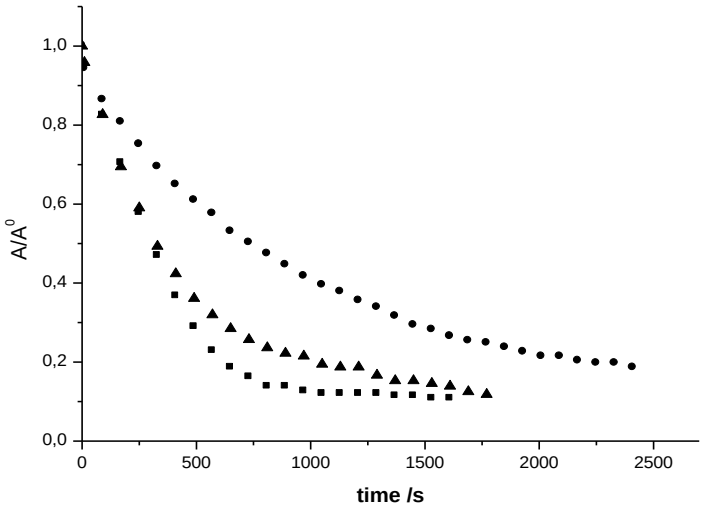

Figure 4
Kinetic profiles of PGR consumption in presence of 3-O-methylgalangin (1.8 mM) before (●) and after (▲) of treatment with octanol. Control experiment (■).

Figure 4 shows that the extraction with octanol drastically reduced the protection afforded to PGR, implying that 3-O-methylgalangin is retained almost completely in the organic phase. These results supports then those obtained by Romero (2010). This author have shown that the presence of micelles decreased significantly the protection of PGR afforded by lipophilic antioxidants (β-carotene, octyl gallate), while no effect of micelles was observed for hydrophilic antioxidants such as Trolox, caffeic acid, gallic acid, and ascorbic acid.

Table 1 shows the ORAC indexes obtained by both resins and its major flavonoid. These indexes were obtained in homogenous phase (ORAC-PGR) and in heterogeneous media. Also is included the ratio between both index (ORAC-PGR/ ORAC-PGR_{MIC}). Values for reference antioxidants have been incorporated for comparison.

Table 1
Indexes ORAC obtained by the resinous extract and 3-O-methylgalangin in absence (ORAC-PGR) and presence of Triton X-100 micelles (ORAC-PGR_{MIC}).*

	ORAC-PGR ± s.d	ORAC-PGR_{MIC} ± s.d	ORAC-PGR/ ORAC-PGR_{MIC} ± s.d
3-O-methylgalangin	0.11 ± 0.03	0.05 ± 0.01	2.2 ± 0.9
H. sinuatum	4.0 ± 0.1	0.37 ± 0.01	10.8 ± 0.1
H. stenophyllum	11.1± 0.1	1.4 ± 0.1	7.9 ± 0.1
Octyl gallate**	9.8 ± 0.3	1.6 ± 0.2	6.1 ± 0.9
Methyl gallate**	12.0 ± 0.4	7.0 ± 0.6	1.7 ± 0.2
Red wine**	36.0 ± 2.0	24 ± 3	1.5 ± 0.3

***Values of the pure compounds are given relatives to Trolox efficiency. Values of the exudates are given in Trolox miliequivalents per gram of dry material. Red wine value is given in Trolox miliequivalents per litter.**
**** Values obtained from Romero (2010).**

The results of the table 1 show that ORAC values of *H.sinuatum* are approximately one-third of those obtained with *H. stenophyllum* in both media. Furthermore, significant differences in the ORAC indexes are observed, in heterogeneous media for both extracts. In fact, the protection of PGR is reduced 91% and 88% with *H. sinuatum* and *H. stenophyllum*, respectively. This large reduction of protection implies that the active components of both extracts are located preferably in the interior of Triton micelles. Furthermore, a comparison of the ratio for 3-O-methylgalangin (2.2) and those of the extracts allows concluding that this flavonoid marginally contributes to the antioxidant activity of the extracts. In fact, a simple calculation based on the efficiency of 3-O-methylgalangin (Table 1) and its amount present in the exudates indicates that this compound contributes with ca. 1 % to the total antioxidant capacity of the exudates.

Romero (2010) has proposed that the ratio ORAC-PGR/ORAC-PGR$_{MIC}$ represents the degree of hydrophobicity of the antioxidants in study. For example, the ratio ORAC-PGR/ORAC-PGR$_{MIC}$ of octyl gallate (6.1 ± 0.9) in relation to methyl gallate (1.7 ± 0.2) is greater than 3. This means that a greater degree of hydrophobicity, allows a greater addition to the micelle, which is reflected in a reduced protection of PGR consumption.

If ORAC-PGR values of the extracts considered in the present study are compared with other mixtures, such as red wine, it can be concluded that the ratio of ORAC indexes between homogeneous and heterogeneous media are greater for the resinous extracts. This implies that the active compounds of red wine are less hydrophobic than the active components present in resinous extracts.

3-O-methylgalangin, the major flavonoid in the resinous extracts, presents a low reactivity towards peroxyl radicals. This conclusion is similar to that reached regarding its reactivity towards DPPH radicals (Lissi *et al*., 1999) and can be explained in terms of the lack of HO groups in the B ring and the presence of only meta groups in the A ring. The ratio of ORAC values obtained for 3-O-methylgalangin (2.2 ± 0.9) is close to that reported for propyl gallate (1.9) by Romero (2010). This implies a rather high hydrophobicity compatible with the almost total extraction by octanol (Figure 4).

CONCLUSION

The extracts obtained from resinous exudates are able to trap peroxyl radicals. The antioxidants present in these extracts are of high hydrophobicity, as assessed by the ratio between ORAC-PGR values determined in buffer and in the presence of Triton X-100 micelles. 3-O-methylgalangin, the most abundant flavonoid in the extracts presents low reactivity and only marginally contributes to the total antioxidant capacity of the exudates. The results obtained emphasize the importance that has, in microheterogenous systems, the distribution of the antioxidants between the different microenvironments.

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