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Study of bioactive compounds from plants of *Rosmarinus officinalis* L. with antioxidant activity

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INTRODUCTION

Secondary metabolites present in aromatic and medicinal plants are an important source of products with pharmacological interest, and among them, phenols, which are also in animal tissues due to plant ingestion (Shahidi and Naczk, 2004). Those compounds are synthesized as mechanisms of plant defense, and their identity and moreover, their mechanisms of action, in many cases are not known. The elucidation of their biological actions is what allows to define the therapeutic applications of the natural compounds. Furthermore, there is a world tendency of replacing synthetic compounds with antioxidant activity (AOX), such as butylhydroxytoluene (BHT) and butylhydroxyanisol (BHA) due to their harmful effects on health (Storz, 2005).

The general aim of the work is to know the biological actions of plant bioactive compounds of the Lamiaceae family in order to evaluate their potential uses. Historically, studies on antioxidants (AOX)were focused in the knowledge of the production and regulation of them, and virtually other biological properties were ignored. The bioactive properties of rosemary (*R. officinalis*) are mainly attributed to the high content of phenols, one of the main compound groups non-essential for diet. In this work active compounds of the terpene class are investigated by a combination of chemical methods and bioassays.

METHODOLOGY

Determination of the AOX activity. Measurement of the free radical scavenging capacity by the DPPH assay, and bleaching of *beta*-carotene of plant extracts and pure compounds. The extraction, identification and quantity analysis of the studied compounds were performed according to Moreno *et al.* (2006). The microbicide activity was carried out by the plate diffusion method and by the broth dilution technique. The minimum inhibitory concentrations (MIC) and the minimum bactericide concentrations (MBC) were determined. A range of ~ 10^3-10^4 colonies formed units (CFU) per ml was used. The effect on viability, proliferation and cell cytotoxicity of the plant extracts was studied in cell lines of fibroblasts.

RESULTS AND DISCUSSION

We have previously reported a high diterpene content in extracts of rosemary plant cultured in northwestern Argentina (Moreno *et al.*, 2006). With the purpose of developing natural alternatives for industry the AOX activity of rosemary extracts (RE) was compared with other synthetic and natural AOXs of commercial use (Fig 1).

The Fig. l shows that the AOX activity of the rosemary extracts, and main compounds, such as the rosmarinic acid (RA) and the carnosic acid (CA) showed an activity comparable with that of BHT and *alpha*-tocopherol. Also, the AOX activity was investigated in mixtures in order to evaluate the type of interaction (Fig. 2 and 3).

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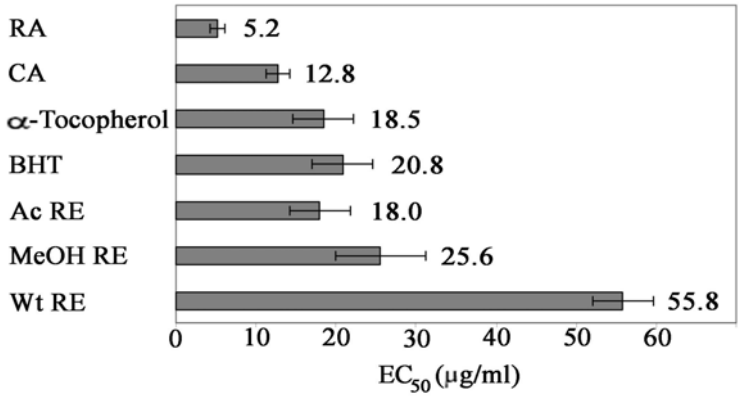

Figure 1. AOX activity of rosemary extracts in comparison with commercial antioxidants.

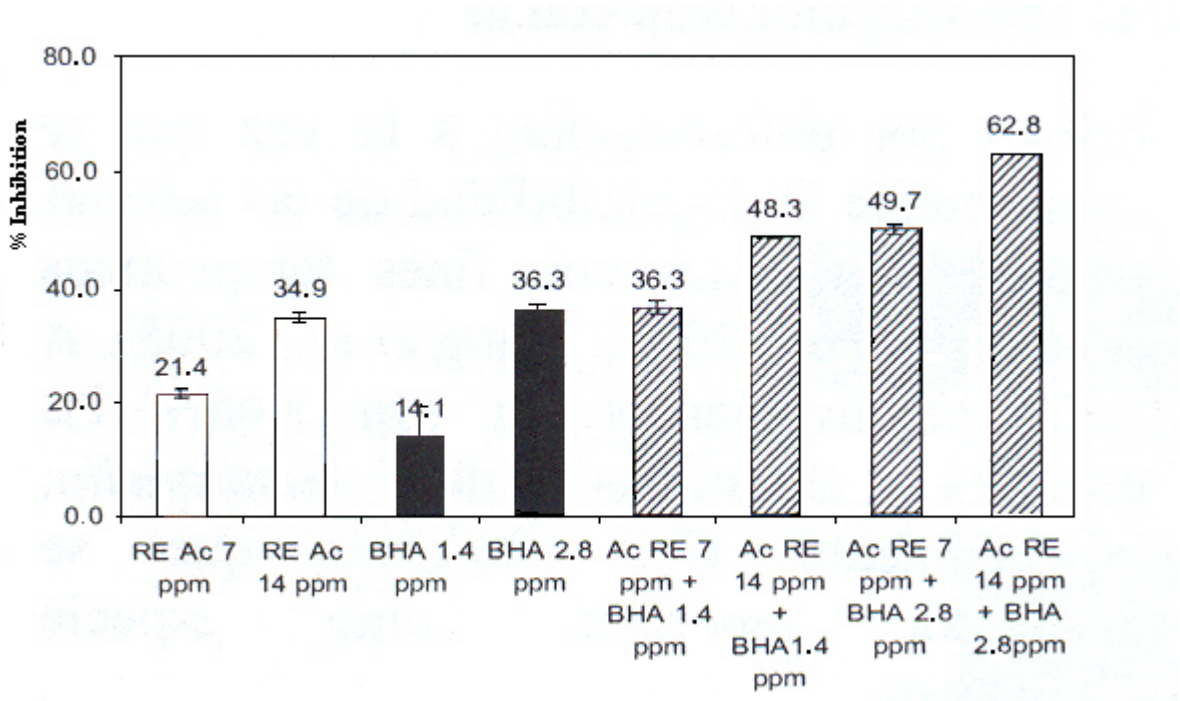

Figure 2. Free radical scavenging activity of rosemary extracts, BHA, and mixtures. Activity is expressed like the percentage DPPH inhibition.

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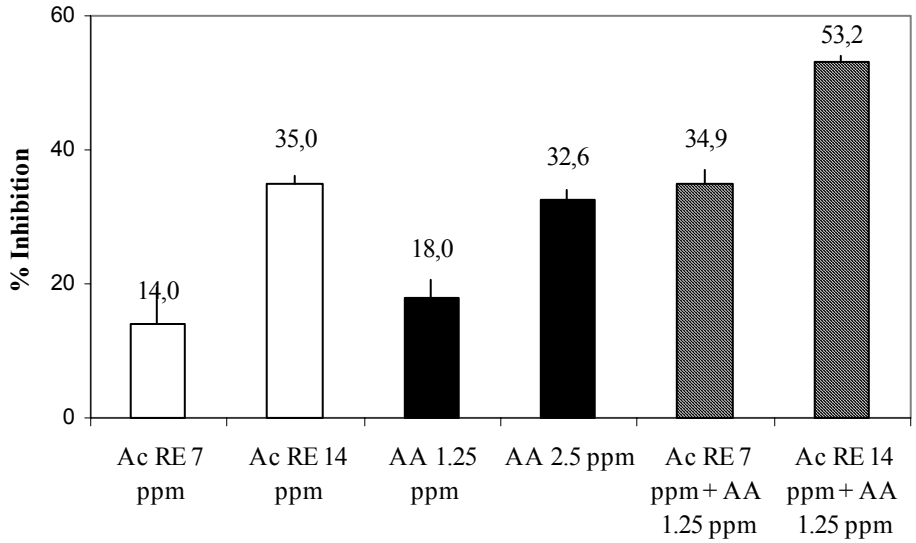

Figure 3. Free radical scavenging activity of rosemary extracts, ascorbic acid (AA), and mixtures.

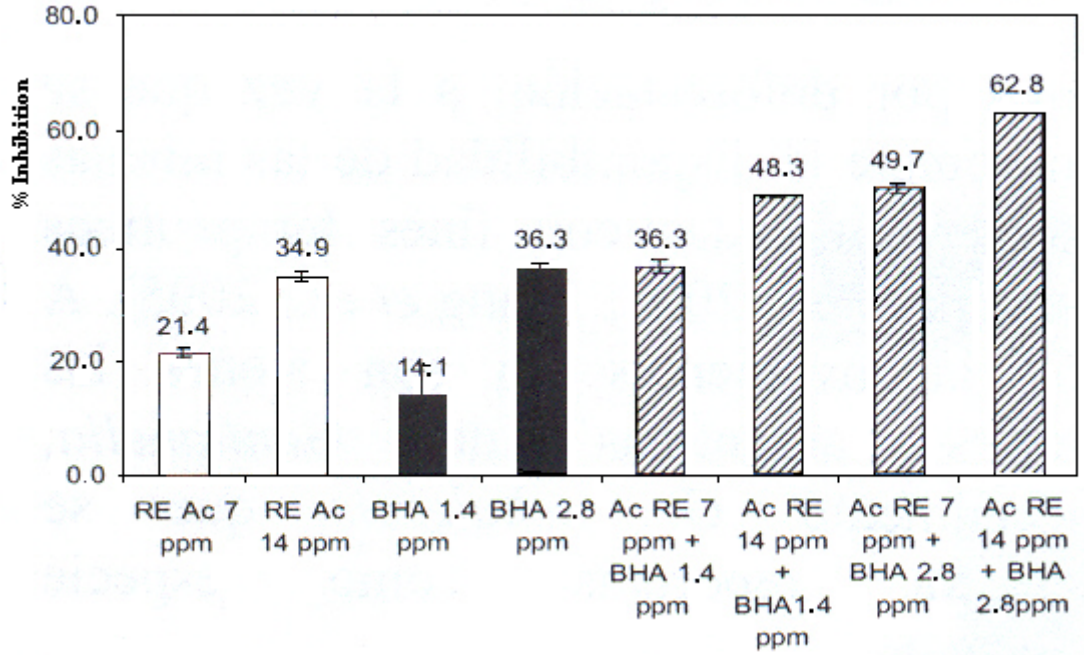

Figure 4. Comparison of the antimicrobial activity of rosemary extracts with BHA, BHT and benzoic acid. The 0% inhibition accounts for *E. coli* culture without inhibitors.

RE shows a positive interaction with BHA and ascorbic acid, respectively (Fig. 2 and 3). This effect, in general, is observed with the other methods (in aqueous media as lipophilic, not shown data).

We have previously reported that RE besides its AOX activity shows an important antimicrobial activity. Then, we studied that activity in comparison with other commercial compounds. Fig. 4 showed

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that 400 ppm of rosemary extracts have an antimicrobial activity similar to 250 ppm of BHA. By other experiments we know that RE has a positive interaction with BHA.

We are studying the type of interaction with other commercial compounds on different bacteria of food and clinical interest. The mechanism of action of plant compounds as well as their toxicity and effects on viability and cell differentiation is being investigated *in vitro* in cell models and in animals.

CONCLUSIONS

It was demonstrated that plant extracts shows similar activities than commercial compounds (ascorbic acid, vitamin E, BHA and BHT) and exhibited positive interactions in combination with them. These results are fundamental for their potential industrial application.

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