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extracts of Mentha cervina from Portugal

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INTRODUCTION

MATERIALS & METHODS

Mints (*Mentha* spp.) is an aromatic plant used in food industry, for flavouring, in perfumery and for pharmaceutical preparations. Some species of this genus are used in folk medicine as antispasmodic, choleretic, carminative and its essential oils are used as an anti-inflammatory, and, since antiquity, it has been known to have antimicrobial proprieties. *Metha cervina* (L.) Opiz (Lamiaceae), is a type of mint that grows wild in river banks, in the north-western regions of Iberian Peninsula. It is an aromatic plant used for culinary purpose, especially to aromatize fish dishes, and for its medicinal properties, infusions with digestive properties (Gonçalves *et al.* 2007; Politi *et al.* 2008). Antioxidant are frequently found in plants, mostly in herbs. They are defined as, "compound which, when present at a concentrations much lower than those oxidisable substract(s), delays to a significant extent, or even inhibits, oxidation of the substract" (Gião *et al.* 2007b). Antioxidants have been employed in food industry, for their ability to preserve food, and the plant extract containing mainly phenolic compounds (antioxidants), are being used for their beneficial health effects (Gião *et al.* 2007a). The main objective of this work was to analyze the antimicrobial and antioxidant activity of *Mentha cervina* extracts in order to assess its potential for the development of functional ingredients that may be applied in the food or cosmetic industries.

Plant extracts :

The tested samples in this work were tinctures obtained from different mixtures of ethanol/water (100%, 65 %, 40 %, 20 % and 0 %(v/v)), infusions and essential oil (hydrodistillation)

Antimicrobial activity :

Screening for antimicrobial activity



Antioxidant activity :

Total antioxidant capacity and total phenol content assay, according to Gião et al. (2007a)



Minimum inhibitory concentration (MIC) determination



Protection of DNA from degradation, according to Gião et al. (2007b)



RESULTS & DISCUSSION

- Tinctures and infusions did not show any antimicrobial activity at concentration range of $40 mg\ ml^{-1}$ and $80 mg\ ml^{-1}$.

 Essential oil inhibited all the tested microorganisms by disc diffusion assays. The extract showed the highest activity towards *Candida albicans* and the lowest upon *Listeria innocua* and *S. aureus*, as shown by halo diameter (Table 1).

* Results of MIC showed that the efficacy of essential oil was higher for *Candida albicans* and *E. coli* (Table 1) with the lowest MIC (5 µl ml¹). Supporting the disc diffusion test the extract showed the lowest activity against *S. aureus* and *Listeria innocua*.

• The antioxidant capacity (Table 2) of tinctures and infusions determined by the ABTS+ method ranged from 0,571 \pm 0,017 for 65% tincture, down to 0,044 \pm 0,005 ascorbic acid equivalent (g L-1) for aerial part infusion. The phenolic acids contents range from 0,724 \pm 0,025 for 65% tincture, down to 0,011 \pm 0,002 gallic acid equivalent (g L-1) for aerial part infusion. The antioxidant capacity of essential oil was determined by TEAC method and showed a high value (0.941 \pm 0.033 TROLOX equivalent (g L-1)).

 The extracts of tinctures showed effective protection by DNA assay (Table 2) for 100%, 65%, 40%, 20% and 0% of ethanol/water mixtures at 40 mg/ml and absence of protection for essential oil at 40 mg/ml. No extract revealed pro-oxidant activity (Fig. 1)



Table 1. Antimicrobial activity (MIC) of M. cervina essential oil

Strains	Halo diam. (mm)*	MIC (µ1 ml ⁻¹)		
Escherichia coli NCTC 9001	11	5 µl ml ⁻¹		
Salmonella spp ATCC 3076	10.5	10 µl ml ⁻¹		
Staphylococcus aureus NCTC 8532	10	40 µ1 ml-1		
Staphylococcus aureus ATCC 29213	10	40 µl ml ⁻¹		
Bacillus cereus ATCC 11778	10.5	10 µl ml-1		
Listeria innocua NCTC 11286	10	80 µl ml-1		
Candida albicans (clinical isolate)	13.5	5 ul ml ⁻¹		

- Disc diffusion assay



1 2 3 4

Fig 1. Electrophoretogram from DNA assay (positive control, lanes 1; negative control, lanes 2; antioxidant effect of sample, lanes 3; sample effect, lanes 4).

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Table 2. Total antioxidant capacity, total phenol content (average ± standard deviation) and anti- and pro-oxidant effects on DNA (malitative) of different extracts

Sample		ABTS ^{*+} (g L ⁻¹ ascorbic acid equivalent)	Folin–Ciocalteu (g L ⁻¹ gallic acid equivalent)	Protection of DNA from Degradation						
				40 mg/ml of sample		60 mg/ml of sample		80 mg/ml of sample		
				А	Р	А	Р	А	Р	
Aerial Parts ïnctures	100% Ethanol	0.096 <u>+</u> 0.008	0.204 <u>+</u> 0.030	+	-					
	65% Ethanol	0.571 <u>+</u> 0.017	0.724 <u>+</u> 0.025	+	-					
	40% Ethanol	0.423 ± 0.023	0.474 ± 0.063	+	-	ND		ND		
	20% Ethanol	0.270 <u>+</u> 0.012	0.341 ± 0.021	+	-					
	0% Ethanol	0.259 <u>+</u> 0.033	0.247 ± 0.017	+	-					
nfusion	Powder	0.160 <u>+</u> 0.009	0.154 <u>+</u> 0.003	-	-	+ -				
	Aerial Parts	0.044 <u>+</u> 0.005	0.011 <u>+</u> 0.002	-	-	•		+	•	
Essential Oil		TEAC (g L-1 TROLOX equivalent)*		А	Р	Α	Р	Α	Р	
		0.941 <u>+</u> 0.033		-	-		-	-		

Note: - no effect, + effect, ND no data A, antioxidant; P, pro-oxidant

TEAC, TROLOX equivalent antioxidant capacity * (method improved in our laboratory)

CONCLUSION

 Essential oil showed antimicrobial activity against a broad group of microorganisms (Gram negative and positive bacteria and yeasts).

• The 65% tincture showed the best antioxidant activity via both assay and did not reveal pro-oxidant activity