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Artigo

Preliminary study of the antimicrobial activity of *Mentha x villosa* Hudson essential oil, rotundifolone and its analogues

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RESUMO: "Estudo preliminar da atividade antimicrobiana do óleo essencial de *Mentha x villosa* Hudson, rotundifolona e seus análogos". Os óleos essenciais apresentam atividade antimicrobiana contra uma variedade de bactérias e fungos, incluindo espécies resistentes a antibióticos e antimicóticos. Neste contexto, este trabalho objetiva a avaliação da atividade de antimicrobiana do óleo essencial de *Mentha x villosa* Hudson (hortelã-da-folha-miúda) - seu componente majoritário (rotundifolona) e quatro análogos sintéticos da rotundifolona (epóxi-limoneno epóxi-pulegona, epóxi-carvona e (+)-pulegona) frente a cepas padrão de *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 76645 e uma cepa de *Staphylococcus aureus* meticilina - resistente - MRSA (171c) de clínica humana. Como método, foi utilizada a difusão em placas com médio sólido. Os resultados mostraram que o óleo de *Mentha x villosa*, rotundifolona, epóxi-limoneno e (+)-pulegona, são semelhante em função da atividade antimicrobiana para as cepas de *S. aureus* e *C.albicans* testadas. Todos os produtos apresentaram potencial antimicrobiano com atividade antibacteriana para *S. aureus* ATCC 25923 e atividade antifúngica para *C. albicans* ATCC 76645. Nenhum dos produtos apresentou atividade antimicrobiana para as cepas de *E. coli* ATCC 25922 e *P. aeruginosa* ATCC 27853, representantes das bactérias Gram negativas.

Unitermos: *Mentha x villosa*, Labiatae, óleo essencial, rotundifolona, atividade antimicrobiana.

ABSTRACT: Essential oils present antimicrobial activity against a variety of bacteria and yeasts, including species resistant to antibiotics and antifungicals. In this context, this work aims at the evaluation of the antimicrobial activity of the essential oil of *Mentha x villosa* Hudson ("hortelã da folha miúda"), its major component (rotundifolone) and four similar analogues of rotundifolone (limonene oxide, pulegone oxide, carvone epoxide and (+)-pulegone) against strain standards of *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 25922, *Pseudomona aeruginosa* ATCC 27853, *Candida albicans* ATCC 76645 and one strain of meticilin – resistant *Staphylococcus aureus* - MRSA (171c) from human clinic. The method of the diffusion in plates with solid medium was used. The results showed that the oil of *Mentha x villosa*, rotundifolone, limonene oxide and (+)-pulegone, are similar regarding the antimicrobial activity against the tested strains of *S. aureus* and *C. albicans*. All of the products present antimocrobial potential with antibacterial activity for *S. aureus* ATCC 25923 and antifungal activity for *C. albicans* ATCC 76645. None of the products presented antimicrobial activity for the strains of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, representatives of the Gram negative bacteria.

Keywords: *Mentha x villosa,* Labiatae, essential oil, rotundifolone, antimicrobial activity.

INTRODUCTION

The use of the plants with medicinal purposes is as old as humanity's history. People, throughout the time used plants as a form to obtain relief of the symptoms or the cure of their diseases. Either as a religious, or as a healing process, they highlighted the aromatic plants holders of essential oils, whose components and biological activities became objective of several researches in the last years (Cowan, 1999; Hoareau; Da Silva, 2001; Agostini et al., 2005; Silva-Santos et al., 2004; Bieski, 2005).

Essential oils present antimicrobial activity against a variety of bacteria and yeasts, including resistant species to antibiotics and antifungal agents (Carson et al., 1995; Duarte et al., 2004; Bertini et al., 2005; Oliveira et al., 2006; Lima et al., 2006). Composition of the essential

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oils can vary with the climate, geographical area, seasons, soil conditions, crop period and extraction technique (Maciel et al., 2002; Bertini et al., 2005; Carvalho-Filho et al., 2006; Potzernheim et al., 2006).

Several species of mentha have been investigated because of the essential oil produced by their leaves (Hiruma, 1993; Monte et al., 2001; Gobert et al., 2002; Lorenzo et al., 2002; Marchese et al., 2005; Sartoratto et al., 2004; Bertini et al., 2005; Bieski, 2005). The genus *Mentha*, family Labiatae, of the subfamily Nepetoidae and of the tribe Mentheae, is composed by approximately, 25 species. *Mentha x villosa* is an herb cultivated throughout Brazil and is used as traditional medicine in the treatment of amebiasis, giardiasis and schistosomiasis (Hiruma, 1993; Matos et al., 1999; Monte et al., 2001). The essential oil and rotundifolone from *Mentha x villosa* showed vasorelaxant effect (Guedes et al., 2004a, Guedes 2004b).

No-rational use of antibiotics has been causing a series of problems for the enviroment and for the human beings, among which stands out the appearance of resistance. Since the beginning of the eighties it is observed that the antimicrobial agents number decreased considerably, while the resistance of the microorganisms to them has been growing in a fast way due to the development of new resistance mechanisms (Moellering-Jr, 2000).

For this reason, this work aims at the evaluation of the antimicrobial activity of the essential oil of *Mentha x villosa* ("hortelã da folha miúda"), its major component (rotundifolone) and four analogues of rotundifolone (limonene oxide, pulegone oxide, carvone epoxide and (+)-pulegone) against standards strain of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomona aeruginosa* ATCC 27853 and one strain of Meticilin – Resistant *Staphylococcus aureus* - MRSA (171c) from human clinic.

MATERIAL AND METHODS

Botanical material

The collection of the leaves of *Mentha x villosa* Hudson (approximately 2 kg), was carried out in the morning in the Medicinal Plants' Garden of the Laboratorio de Tecnologia Farmacêutica of the Universidade Federal da Paraíba - LTF/UFPB (7º08'29''S, 34º50'48'' W).

Extraction of the essential oil

The fresh leaves were subjected to steam distillation using a Clevenger apparatus (Wasicky, 1963). The extraction period lasted an average of six hours and an oil of yellowish coloration and characteristic odor was obtained.

Isolation of rotundifolone from the essential oil of *Mentha x villosa*

The isolation and identification processes followed the methodology in described by Hiruma (1993) and Matos et al. (1999). The essential oil was submitted to preparative thin layer chromatography, using hexane as eluent. When the plates were exposed to UV light (254 nm) it was possible to observe rotundifolone as the major component of the essential oil. Rotundifolone was removed of the chromatographic plates and later recovered of the silica-gel by extraction with chloroform.

Rotundifolone analogues

The substances limonene oxide (Thomas; Bessiere, 1989), pulegone oxide (Katsuhara, 1967), and carvone epoxide (Santos et al., 1997) were prepared as previously described. (+)-Pulegone was purchased from Aldrich.

Microorganism strains

Antimicrobial activity tests were carried ou against the bacteria *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomona aeruginosa* ATCC 27853 and one strain of MRSA (171c) from human clinic and the yeast *Candida albicans* ATCC 76645.

Culture media

Bacteria were assayed in Mueller-Hinton Agar (DIFCO Laboratories) and *C. albicans* on Sabouraud Dextrose Agar (DIFCO Laboratories).

Diffusion method in plates with solid medium

The method used was the diffusion in agar (Bauer et al., 1966) following the recommendations of the NCCLS (Nattional Commitee for Clinical Laboratory Standart).

Inocula

Starting from cultures maintained in Agar Mueller-Hinton, for 24 hours (Bacteria), to the temperature of 37 °C and 24-72 h (fungi) to the room temperature, the inoculate was prepared (Benoudia et al., 1988; Odds, 1989) and standardized in physiologic solution (chloride of sodium) to 0.85% sterile (Casals, 1979; Plempel, 1986). Initially, it was prepared a comparative solution with the one of sulfate of barium of the tube 0.5 of the McFarland and cellular counting in a Newbawer's camera. The suspension contained, approximately, 10^6 UFC/mL (Casals, 1979; Drutz, 1987). The plates containing middle of culture, they were sowed with this inoculate with the aid of sterile swabs.

Evaluation of the antibacterial activity

Approximately 20 mL of the culture medium was added to each disposable sterile plate (90 mm). After solidification, the inocula were dispersed in the surfaces, with the aid of a swab. Afterwards, cavities with 6mm of diameter were made, where 50 µL of each product were placed. The plates were incubated at 37 °C for 24 hours. Each test was carried out in duplicate against each selected strain. The final result was obtained by calculating the arithmetic average of the diameter of the halos of inhibition in the two assays, being considered active against the strains in study, those which produced halos starting from 9 mm of diameter (Gundidza, 1986).

RESULTS AND DISCUSSION

The results obtained during the tests are presented in the table 1.

Several studies indicate that the essential oils of the genus *Mentha* possess biological activity against several bacteria and yeast (Oumzil et al., 2002; Sartoratto et al., 2004; Mounchid et al., 2005). According to Ohno et al. (2003), there are differences in the activity of an oil to another or even of their subproducts, due to the structure activity relationship. Many of the oil components may possess the ability to break or to penetrate the lipid structure present in the Gram-negatives bacteria wall.

Table 1 shows the results found in this study, where one can observe that the essential oil of *Mentha x villosa*, rotundifolone and their analogues did not present any inhibition of the growth on the samples of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. (Figure 1).

Regarding the strains of *Staphylococcus aureus*, it was observed that all of the tested substances presented antibacterial activity, characterized by the presence of zones of growth inhibition with diameters that varied from 9 mm (carvone epoxide) to 16 mm (*Mentha x villosa* essential oil) for the strain *S. aureus* ATCC 25923, while limonene oxide and carvone epoxide did not present any activity against the studied strain (171c). This fact can be justified by the individual behavior of each strain and by the presence of different genes of plasmidial and/or cromossomial resistance that determine, besides the virulence factors, the resistance to the antimicrobian agents (Trabulsi, et al., 1999).

All of the products presented antifungal activity against the strain of *Candida albicans* ATCC 76645, developing halos of inhibition that varied from 15 mm (limonene oxide) to 18 mm (*Mentha x villosa* and carvona epoxide).

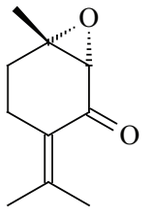
The essential oil of *Mentha x villosa* was the product that presented the largest zones of growth inhibition among the sensible strains. This happens, probably because the essential oils are composed by, several substances that can interact jointly in a synergic/potential form, giving them strong antimicrobial activity (Carson et al., 1995; Mounchid et al., 2005). Other factor that should also be studied and considered is related to the diffusion ability of the tested product in the culture medium, which can favor in some situations, the diameter of the zones of growth inhibition.

The results of this preliminary study in relation to the antimicrobial structure-activity of rotundifolone and their analogues, showed that rotundifolone, pulegone oxide and (+)-pulegone presented similar antimicrobial activity, diverging discreetly just in relation to the size of the zones of growth inhibition, what may be considered irrelevant in function of the presence of activity proven by the existence of growth inhibition zones ≤ 10 mm (Gundiza, 1986).

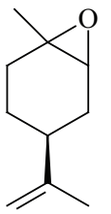
The presence of a ketone ring in the molecule carvone epoxide in a different position of the original molecule rotundifolone, as well as the presence of a single epoxide group and the absence of the ketone in the molecule of limonene oxide can be factors that interfere with the antimicrobial activity, when appraised in function of the size of the growth inhibition zones. However, the antimicrobial activity of a product can not be determined only in function of the presence/absence of zones of growth inhibition, or also as a function of its size. Other microbiologic subsequent studies such as evaluation of the effect of those products on the microbial kinetics, are being carried out and they will probably help to elucidate the real antimicrobial potency of these products, and also define the effects as bactericide/fungicide or bacteriostatic/fungistatic.

Table 1. Effects of the essencial oil of *Mentha x villosa* Hudson, rotundifolone and its analogues against the tested strains of the microorganisms.

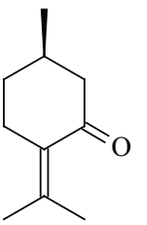
Substances (200 µg.mL)	Tested microrganisms/diameters of inhibition zones (mm)				
	S. aureus ATCC 25923	*S. aureus* MRSA (171c)	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 76645
Mentha x villosa oil	16	13	0	0	18
Rotundifolone	14	12	0	0	17
Limonene oxide	9	0	0	0	15
Pulegone oxide	12	12	0	0	17
Carvone epoxide	9	0	0	0	18
(+)-Pulegone	11	12	0	0	16



Rotundifolone



Limonene oxide



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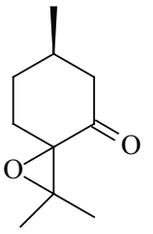
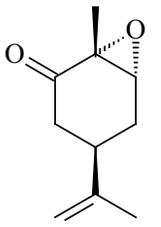


Pulegone oxide



Carvone epoxide

Figure 1. Compounds used in this study.

CONCLUSION

According the found results, it can be concluded that the essential oil of *Mentha x villosa*, rotundifolone, limonene oxide and (+)-pulegone, are similar in terms of the antimicrobial activity on the tested strains of *S. aureus* and *C. albicans*.

All of the products present antimocrobial potential with antibacterial activity for *S. aureus* ATCC 25923 and antifungal activity for *C. albicans* ATCC 76645.

Limonene oxide and carvone epoxide were the substances which presented the smallest antimicrobial potentials.

None of the products showed antimicrobial activity for the strains of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, Gram negative bacteria.

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