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Phytochemical Prospection, Toxicity and Antimicrobial Activity of *Mentha arvensis* (Labiatae) from Northeast of Brazil

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ABSTRACT

The present study has been designed with the objective to examine the ethanol extract of *Mentha arvensis* leaves, in order to evaluate its chemical composition, investigate its in vitro antimicrobial potential against strains of *Escherichia coli, Pseudomonas aeruginosa, Shigella flexineri, Klebsiella pneumoniae* and *Sthaphylococcus aureus*, and find its toxicity toward Artemia salina and. Phytochemical analysis revealed the presence of catechic tannins, flavones, flavonols, xantones, flavonols, flavonones and steroids. The antibacterial activity is more significant against *Sthaphylococcus aureus*. In toxicity tests on Artemia salina, the ethanol extract from Mentha arvensis leaves showed LC_{50} value of 100 µg/mL. These results may justify the popular use of this species as it has antimicrobial activity and demonstrate its significant toxic effect on brine shrimp. However, in order to evaluate possible clinical application in therapy of infectious diseases, further studies about the safety and toxicity of isolated compounds are needed.

Key words: Antibacterial activity, *Mentha arvensis*, phytochemical prospection, toxicity

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INTRODUCTION

The genus *Mentha* (Labiatae) is originated from Asia. The *Mentha* species are herbaceous plants, mainly cultivated for their aromatic, condimentary and medicinal properties.[1] *Mentha arvensis* is a herbaceous plant growing to 40 cm height and is an aromatic herb with strong refreshing odour. It is popularly known as hortelã-vick. In traditional medicine, it is indicated as stomachic, carminative, nasal decongestant and used against skin infections.[2]

The essential oil of this species is rich in menthol (70%), which is used in nasal inhalants, perfumes, cigarettes and pharmaceutical industries.[1] Some compounds identified in *Mentha arvensis* were menthol, α-terpineol, *p*-menthone, menthol acetate and others.[3,4]

Plants have been used for many years as medicines by population, providing good sources of pharmacologically actives substances and improving the therapeutic arsenal. However, many plants are known to be toxic. Therefore,

researches are being carried out to determine their pharmacological action and toxicity.[5]

The purpose of this work is to realize the chemical prospection and evaluate the antimicrobial and toxicological activities of ethanolic extract of *M. arvensis* leaves.

MATERIALS AND METHODS

Plant material and ethanolic extract obtention

Fresh leaves of *M. arvensis* collected from the Medicinal and Aromatic Plant Garden at Pimenta Campus of Regional University of Cariri (URCA), Crato, CE, Brazil, in May 2008. A voucher specimen (#4019) was deposited in the Herbário Caririense Dárdano de Andrade Lima of the same University.

The ethanolic extract was obtained using 95% ethanol as a solvent and reflux in Soxhlet apparatus until exhausted. The *Menta arvensis* extract was then dried (yield 5.8%).

Phytochemical prospection

The phytochemical tests to detect the presence of heterosides, saponnins, tannins, flavonoids, steroids, triterpens, cumarines, quinones, organic acids and alkaloids were performed following the method described by Matos.[6] These tests were based on the visual observation of color modification or precipitate formation after the addition of specific reagents.

Antimicrobial activity evaluation

Antimicrobial activity of extract from *Mentha arvensis* was tested against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), *Shigella flexineri* (ATCC 12022), *Klebsiella pneumoniae* (ATCC 10031) and *Sthaphylococcus aureus* (ATCC 12692) by using the agar diffusion method.[7] The bacterial lines were cultivated in Brain Heart Infusion media (BHI) and incubated at 37° C for 24 h. After this period, they were replicated on Petri dishes containing Muller–Hilton (MH) agar. The plates containing the microorganisms were then perforated and the cavities were filled with 25 µL of the extract solutions at 10, 5, 2.5, 1.25, 0.6 and 0.3% concentrations.

Trials were performed in triplicate and commercial antibiotic disks of chloramphenicol (30 µg/disk) and tetracycline (30 µg/disk) were employed as positive controls, while ethanol was served as negative control. Inhibition halos were measured 24 h after initial exposure. The results were expressed in terms of the diameter of the inhibition zone: < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.[8,9]

Brine shrimp cytotoxicity assay

Artemia salina encysted eggs were incubated in artigical seawater under light at 28°C. After incubation for 24 h, nauplii were collected with a Pasteur pipette and kept for an additional 24 h under the same conditions to reach the metanauplii stage. The samples (triplicate) to be assayed were dissolved in Tween 80 and were diluted serially (1000, 250, 125, 100, 75 µg/mL) in seawater. Ten nauplii were added to each set of tubes containing the samples. Controls containing Tween 80 in seawater were included in each experiment. $K_2Cr_2O_7$ was used as a positive control. After twenty-four hours, the number of survivors was counted.[10]

Lethal concentration determination

The lethal concentrations of plant extracts resulting in 50% mortality of the brine shrimp (LC_{50}) and 95% confidence intervals were determined from the 24 h counts and the dose-response data were transformed into a straight line by means of a trendline fit linear regression analysis; the LC_{50} was derived from the best-fit line obtained.

RESULTS AND DISCUSSION

Phytochemical prospection of *M. arvensis* leaves ethanolic extract indicated the presence of different secondary metabolites classes [Table 1]. Many of them are known to have different therapeutic applications. For example, tannins possess antibacterial, antiviral, moluscicidal and antitumoral properties.[11-14] While flavonoids, also present in *M. arvensis* extract, is recognized to have anticancer, antiviral and antihemorrhagic properties.[15]

The larval mortality index obtained on toxicity bioassay

Table 1: Phytochemical screening of *M. arvensis*

Secondary metabolite	Result
Tannins	Positive
Flavones	Positive
Flavonol	Positive
Xantones	Positive
Flavanonols	Positive
Flavanones	Positive
Alkaloids	Negative
Steroids	Positive
Triterpenes	Negative

Table 2: Antimicrobial activity of the ethanolic leaf extract of *M. arvensis*

Microorganisms	Concentration of the extract (%)						$C+_1$	$C+_2$	C-
	10	5	2.5	1.25	0.6	0.3			
E. coli	12±0.1	11±0.3	10±0.7	9±0.8	8±0.1	7±0.1	9±0.4	NA	NA
S. aureus	21±0.7	19±0.8	17±0.5	13±0.4	12±0.7	9±0.7	18±0.5	22±0.1	NA
P aeruginosa	11±0.1	10±0.7	9±0.4	8±0.2	7± 0.2	NA	NA	NA	NA
K. pneumoniae	13±0.7	12±0.7	10±0.7	9±0.6	8±0.7	7±0.3	18±0.7	NA	NA
S. flexineri	14±0.7	13±0.6	10±0.1	9±0.1	8±0.6	7±0.7	23±0.1	14±0.1	NA

$C+_1$ (Chloramphenicol); $C+_2$ (Tetracycline); C- (Ethanol); NA (No activity).

ranged from 0 to 100%. The LC_{50} found was 100 μg/mL, indicating significant cytotoxic activity against *Artemia salina.* According to other studies, the potential use of some botanical fractions as larvicidals confirms that *M. arvensis* extract results obtained here are comparable to those found in the literature.[16,17]

As shown in Table 2, the ethanolic extract obtained from *M. arvensis* inhibited the growth of the bacterial strains; *Sthaphylococcus aureus* (Gram positive, inhibition zone diameter mean 21 mm) was the most susceptible of all the test microorganisms in a similar way to that of tetracycline. This result indicates that *M. arvensis* extract has an antibacterial potential similar to that of a commercial drug. No significant differences were detected between the *E. coli*, *P. aeruginosa*, *S. Flexineri* and *K. pneumoniae* inhibition zone diameters.

According to the parameters found in literature,[8,9] *M. arvensis* extract was classified as "very active" against *S. aureus,* "active" against *E. coli*, *K. pneumonia*e and *S. flexineri* and "partially active" against *P. aeruginosa*.

CONCLUSION

The chemical prospection *of M. arvensis* extract detected the presence of flavonones, flavonols and tannins. The antibacterial activity might be related to the presence of tannins as these metabolites have proved antibacterial properties.

M. arvensis was classified as toxic to *Artemia salina* as the observed LC_{50} was minor than standard limit (1000 μg/mL). The antibacterial evaluation revealed that *M. arvensis* extract was active against all bacteria strains, being classified as "very active" against *S. aureus*. The results obtained here demonstrate the high biological potential of *M. arvensis*.[18]

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REFERENCES

1. Garlet TM. Produtividade, teor e composição do óleo essencial de espécies de Mentha L. (Lamiaceae) cultivadas em Hidroponia com variação de potássio. Universidade Federal de Santa Maria. Santa Maria, RS: 2007.
2. Matos FJ. Plantas medicinais. Fortaleza, Editora UFC, 2nd ed. 2000.
3. Deschamps C, Zanatta JL, Roswalka L, Oliveira MC, Bizzo HR, Alquini Y. Densidade de tricomas glandulares e produção de óleo essencial em Mentha arvensis L, Mentha x piperita L. e Mentha cf. aquatica L. Ciência e Natura, UFSM 2006;28:23-34.
4. Brito AM. Avaliação da atividade antileishmanial dos óleos essenciais das plantas Cymbopogon citratus (DC) Stapf, Eucalyptus citriodora Hook, *Mentha arvensis* L, e Mentha piperita L. Dissertação do programa de pós-graduação em Saúde e Ambiente da Universidade Tiradentes. 2007.
5. Costa D., Matias, W N,ima, I. O Xaer AL, Costa VBM, Diniz, M. F. F M, Agr, isndary metabolites from Herissantia crispa L(Brizicky) and the toxicity activity against Artemia salina Leach. Quim Nova 2009;32: 48-50.
6. Matos FJ. Introdução à Fitoquímica Experimental. Fortaleza, Edições UFC, 2 ed. 1997.
7. Romeiro R.. (2todos em Bacteriologia de Plantas. Viçosa, Editora UFC. 2001.
8. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol 1998;23:127-49.
9. Alves TM, Silva AF, Brandão M, Grandi TS, Smânia EF, Junior AS, *et al*. Biological screening of Brazilian medicinal plants. Memórias do Instituto Oswaldo Cruz 2000;93:367-73.
10. Meyer BN, Ferrigne NR, Putnam JE, Jacobsen LB, Nichols DE, Mclaughlin JE. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 1982;45:31-4.
11. Scalbert A. Antimicrobial properties of tannins. Phytochemistry 1991;30:3875-83.
12. Okuda T, Yoshiba T, Hatano T. Classification of oligomeric hydrolysable tannins and specificity of their occurrence in plants. Phytochemistry 1993;32:507-21.
13. Marston A, Hostettmann K. Plant molluscicides: Review. Phytochemistry 1985;24:639-52.
14. Okuda T, Yoshiba T, Hatano T. Ellagitannins as active constituents of medicinal plants. Planta Med 1989;55:117-22.
15. Simões CM, Schenkel EP, Gosmann G, Mello JC, Mentz LA, Petrovick PR. Farmacognosia: da planta ao medicamento. Porto Alegre/ Florianópolis, Ed. Universidade/ UFRGS/ Ed. da UFSC, 4 ed. 2002.
16. Simas NK, Lima EC, Conceição SR, Kuster RM, Oliveira FA. Produtos naturais para o controle da transmissão de dengue-atividade de Myroxylon balsamum (óleo vermelho) e de terpenóides e fenilpropanóides. Química Nova 2004;27:46-9.
17. Silva HH, Silva IG, Santos RM, Filho ER, Elias CN. Atividade larvicida de taninos isolados de Magonia pubescens St. Hil. (Sapindaceae) sobre Aedes aegypti (Diptera, Culicidae). Revista da Sociedade Brasileira de Medicina Tropical 2004;37:396-9.
18. Costa JG, Rodrigues FF, Angélico EC, Pereira CK, Souza EO, Caldas GF, *et al*. Composição química e avaliação da atividade antibacteriana e toxicidade do óleo essencial de Croton zehntneri (variedade estragol). Revista Brasileira de Farmacognosia 2008;18:583-6.

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