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BRIEF COMMUNICATION

ANTIFUNGAL ACTIVITY OF *Cymbopogon nardus* (L.) Rendle (CITRONELLA) AGAINST *Microsporum canis* FROM ANIMALS AND HOME ENVIRONMENT

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SUMMARY

Dermatophytosis is a common zoonosis in urban centers. Dogs and cats have played an important role as its disseminators. Environmental decontamination is essential for the prevention of its propagation to humans and animals. However, sanitizers or disinfectants with antifungal activity, currently available, have high toxicity. The present study evaluated the *in vitro* effects of an extract of citronella (*Cymbopogon nardus*) on 31 *Microsporum canis* isolates from animals and home environments. Susceptibility tests were performed based on document M38-A2 (2008) of the Clinical and Laboratory Standards Institute with modifications for natural products. Although susceptibility variation was observed between the fungus tested, the concentrations that inhibited the growth of 50 and 90% of the microorganisms were low (19.5 and 78 µg/mL, respectively). Thus, this citronella extract showed potent fungistatic and fungicide activities against *M. canis* isolated from animals and home environments. Therefore, it could be an alternative for dermatophytosis prophylaxis in the home environment.

KEYWORDS: Citronella; *Microsporum canis;* Dermatophytosis; Profilaxy.

Dermatophytosis is a cutaneous mycosis caused by keratinophilic fungi, with high prevalence among adults and children that inhabit tropical regions. Domestic mammals, especially dogs and cats, clearly play a role as dissemination agents[8]. Several authors have linked animal dermatophytosis[2,15] or surfaces contamination in houses to animal infections[13].

The strategic treatment of dermatophytosis in animals should include environmental decontamination[17]. However, the products available present several limitations related to human health, such as toxicity and potential waste accumulation in the environment[14,18]. In addition, few studies have evaluated the activity of disinfectants against zoophilic fungi[7].

In the search for alternative disinfectants, the present study evaluated the *in vitro* effects of an extract of *Cymbopogon nardus* (citronella) on dermatophytes isolated from domestic animals and the environment, in an effort to provide a new perspective in dermatophytosis prophylaxis.

Cymbopogon nardus (L.) Rendle, popularly known as citronella, is a plant from the *Graminae* family. Several authors have demonstrated the *in vitro* antifungal activity of essential oils of *C. nardus,* and other species of the genus *Cymbopogon,* on pathogenic fungi[4,5,9]. Hair and skin samples from dogs and cats with suspected dermatophytosis were collected by scraping the affected animals, in some veterinary clinics in Maringa (two from *Clinica Veterinária do Unicesumar*, nine from *Clínica Saúde Animal*, nine from *Clínica Ponto Cão* and 11 from *Zooloja*), Parana, Brazil. Samples of domestic environments (e.g. floors and carpets) were collected by the carpet method[3]. The samples were grown on Mycosel Agar (Benton Dickinson, Sparks, MD, USA) for seven days at 25 °C. Of the 60 biological samples, including biotic and abiotic samples, 31 fungal isolates were obtained and identified as *M. canis* by micromorphological technique[11]. These samples, and the standard strain of *Trichophyton rubrum* (ATCC 28189), were tested against the hydroalcoholic extract of *C. nardus* to evaluate the *in vitro* antifungal activity. The leaves of *C. nardus* were collected in "Prof. Irenice Silva" medicinal plant garden of the State University of Maringa, PR, Brazil (lat: -24.35 long: -50.583333). Fresh leaves of *C. nardus* were cleaned with compressed air, cut into small pieces and submitted to turbo extraction for 15 minutes with 77 °GL (Gay Lussac) ethyl alcohol in a ratio of 20% (w/w) at room temperature. The extract was filtered, concentrated in a rotoevaporator and, subsequently, lyophilized. Then, the lyophilized extract of *C. nardus* was solubilized in dipropylene glycol at a rate of 10 mg/mL.

Firstly, the fungi were grown in potato dextrose agar for ten days at

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25 °C. Afterward, the fungal structures were detached in sterile saline solution (0.85%). The inoculum concentration was adjusted to 1-5 × 10^4 colony forming units per millilitre (CFU/mL)[12,20] in RPMI 1640 medium (Roswell Park Memorial Institute, Gibco) with L-glutamine without sodium bicarbonate, buffered with MOPS (3-[*N*-morpholino] propanesulfonic acid, 0.165 M, pH 7.2, Sigma) plus 2% glucose.

The susceptibility assay was performed using the microdilution broth method according to document M38-A2 (2008) of the Clinical and Laboratory Standards Institute[1] with some modifications for natural products. Briefly, the extract was tested at concentrations that ranged from 9.7 µg/mL to 5,000 µg/mL. Terbinafine was used as a control (0.002 and 0.008 µg/mL). The reading was performed, by visual observation, after seven days of incubation at 25 °C. The minimum inhibitory concentration (MIC) was considered the lowest concentration that inhibited 100% of fungal growth compared with the control. For both the citronella extract and terbinafine, the MIC was calculated according to the inhibition of growth of 50% (MIC_{50}) and 90% (MIC_{90}) of the microorganisms.

The minimum fungicidal concentration (MFC) was determined by transferring the contents from MIC assay to plates with drug-free Mycosel Agar. The lowest concentration of the extract that inhibited fungi growth in complete medium was considered the MFC.

Ten houses were visited where 11 animals had confirmed cases of dermatophytosis. In eight, it was possible to isolate the pathogen from the environment. In three houses, asymptomatic animals were found with positive microbiological tests for *M. canis*. In four homes, the disease spread to humans or animals.

The MIC of citronella extract against 31 *M. canis* isolates and the control strain ranged from 9.75 to 625 µg/mL. However, in most of the studied samples, both the MIC_{50} and MIC_{90} were low (19.5 and 78 µg/mL, respectively). Only a low percentage of isolates (3.23%) demanded a high concentration of the citronella extract (625 µg/mL) to show fungicidal activity.

The citronella extract, according to the criteria established by SCORZONI *et al*. (2007)[19], had moderate to strong antifungal activity (Table 1); it was strong for most of the *M. canis* samples tested (80.65%). The present data indicate that the fungistatic and fungicidal activities of the citronella extract were identical for most of the *M. canis* isolates tested. In only six isolates, the fungicidal concentration of the extract was slightly greater than the inhibitory concentration.

Our results demonstrated the *in vitro* efficiency of citronella extract on inhibiting *M. canis* obtained from animals and the home environment where these animals lived. Citronella essential oil has been used as an insect repellent and disinfectant[10,16], but the high cost and manufacturing complexity limits its use. However, citronella extract use in controlling dermatophytosis is encouraged by its low cost, easy formula preparation, and accessibility. It is also rich in citronellal and geraniol[10]. The present study indicates a new option of low-cost disinfectants.

In the present study, the terbinafine MIC of 32 isolates ranged from 0.001 to 1 µg/mL, and the MIC_{50} and MIC_{90} were also low (0.001 µg/mL). These *in vitro* results suggest a homogeneous fungi population profile with regard to antifungal susceptibility. The MIC_{90} for terbinafine was low, confirming the results reported by GUPTA *et al.* (2001)[6]. These data may suggest the use of terbinafine for the treatment of dermatophytosis caused by *M. canis*. Nevertheless, the high cost of this product makes its use prohibitive in environmental control.

Table 1
Activity *in vitro* of the citronella extract (CE) and terbinafine (TERB) on 31 isolates of *Microsporum canis*

	Drugs	*M. canis* (31)
[1]MIC range	CE	9.75-625
	TERB	0.001-1
MIC_{50}	CE	19.50
	TERB	0.001
MIC_{90}	CE	78.00
	TERB	0.001
[2]MFC range	CE	9.75-625
MFC_{50}	CE	19.50
MFC_{90}	CE	78.00

[1]Minimum inhibitory concentration - MIC (µg/mL); MIC_{50} and MIC_{90} for drug and extract: MIC capable of inhibiting 50% and 90% of the isolates, respectively. [2]Minimum fungicidal concentration – MFC (µg/mL); The MFC_{50} and MFC_{90} are the MFCs capable of inhibiting 50% and 90% of the isolates, respectively. *Trichophyton rubrum* ATCC 28189 – MIC = 39 µg/mL, MFC = 39 µg/mL.

In conclusion, the citronella extract showed strong antifungal activity, both fungistatic and fungicidal, against isolates of *M. canis*, suggesting its potential use in the control of zoonoses of fungal origin. Further studies should be conducted incorporating this extract in sanitizers for domestic environments where animals live, with the goal of use it in prophylaxis against dermatophytosis carried by pets.

RESUMO

Atividade antifúngica de *Cymbopogon nardus* (L.) Rendle (citronela) contra *Microsporum canis* de animais e ambiente doméstico

A dermatofitose é uma zoonose comum nos centros urbanos. Cães e gatos têm desempenhado um papel importante como seus disseminadores. A descontaminação ambiental é essencial para a prevenção da propagação da infecção em seres humanos e animais. No entanto, desinfetantes ou sanitizantes com atividade antifúngica que estão disponíveis atualmente têm alta toxicidade. O presente estudo avaliou os efeitos *in vitro* de um extrato de *Cymbopogon nardus* (citronela) em 31 *Microsporum canis* isolados de animais e meio ambiente doméstico. Os testes de susceptibilidade foram realizados com base no documento M38-A2 (2008) do Clinical and Laboratory Standards Institute com modificações para os produtos naturais. Embora tenha sido observada variação de susceptibilidade entre os fungos testados, as concentrações que inibiram o crescimento de 50% e 90% dos microrganismos foram baixas (19,5 e 78 µg/mL, respectivamente). Assim, o extrato de citronela mostrou potente atividade fungistática e fungicida contra *M. canis* isolados de animais e

meio ambiente doméstico. Portanto, este extrato pode ser uma alternativa para a profilaxia da dermatofitose no ambiente doméstico.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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REFERENCES

1. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: approved standard. 2nd ed. Wayne: CLSI Publication M38-A2; 2008.

2. Copetti MV, Santurio JM, Cavalheiro AS, Boeck AA, Argenta JS, Aguiar LC, *et al*. Dermatophytes isolated from dogs and cats suspected of dermatophytosis in Southern Brazil. Acta Sci Vet (Porto Alegre). 2006;34:119-24.

3. Drouhet E, Marcel M, Labonde J. Flore dermatophytique des piscines. Bull Soc Franc Derm Syph. 1967;74:719-24.

4. Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C. Anti- *Candida* activity of Brazilian medicinal plants. J Ethnopharmacol. 2005;97:305-11.

5. Guerra Ordóñez M, Rodríguez Jorge M, García Simón G, Llerena Rangel C. Actividad antimicrobiana del aceite esencial y crema de *Cymbopogon citratus* (DC) Stapf. Rev Cubana Plant Med. 2004;9.

6. Gupta AK, Ahmad I, Summerbell RC. Comparative efficacies of commonly used disinfectants and antifungal pharmaceutical spray preparations against dermatophytic fungi. Med Mycol. 2001;39:321-8.

7. Gupta AK, Brintnell W. Ozone gas effectively kills laboratory strains of *Trichophyton rubrum* and *Trichophyton mentagrophytes* using an *in vitro* test system. J Dermatolog Treat. 2014;25:251-5.

8. Hermoso de Mendoza M, Hermoso de Mendoza J, Alonso JM, Rey JM, Sanchez S, Martin R, *et al*. A zoonotic ringworm outbreak caused by a dysgonic strain of *Microsporum canis* from stray cats. Rev Iberoam Micol. 2010;27:62-5.

9. Hernández Díaz L, Rodríguez Jorge M. Actividad antimicrobiana de plantas que crecen en Cuba. Rev Cubana Plant Med. 2001;6:44-7.

10. Koba K, Sanda K, Raynaud C, Nenonene YA, Millet J, Chaumont JP. Activités antimicrobiennes d'huiles essentielles de trois *Cymbopogon* sp. africains vis-à-vis de germes pathogènes d'animaux de compagnie. Ann Méd Vét. 2004;148:202-6.

11. Larone DH. Medically important fungi: a guide to identification. 5th ed. Washington: ASM Press; 2011.

12. Magagnin CM, Stopiglia CD, Vieira FJ, Heidrich D, Machado M, Vetoratto G, *et al*. Antifungal susceptibility of dermatophytes isolated from patients with chronic renal failure. An Bras Dermatol. 2011;86:694-701.

13. Mancianti F, Nardoni S, Corazza M, D'Achille P, Ponticelli C. Environmental detection of *Microsporum canis* arthrospores in the households of infected cats and dogs. J Feline Med Surg**.** 2003;5:323-8.

14. Miller Jr GT. Ciência ambiental. São Paulo: Thomson Learning; 2007.

15. Nweze EI. Dermatophytoses in domesticated animals. Rev Inst Med Trop Sao Paulo. 2011;53:94-9.

16. Oliveira VL, Alves CLD, Cardenes KMVDH, Pereira ML. Ocorrência de *Microsporum canis* em felinos sadios atendidos no hospital veterinário da unidade 3 da faculdade Anhanguera de Campinas. Anuário da Produção de Iniciação Científica Discente. 2011;13:57-66.

17. Rochette F, Engelen M, Vanden Bossche H. Antifungal agents of use in animal health-practical applications. J Vet Pharmacol Ther. 2003;26:31-53.

18. Schvartsman S. Produtos químicos de uso domiciliar: segurança e riscos toxicológicos. São Paulo: Ed. Almed; 1988.

19. Scorzoni L, Benaducci T, Almeida AMF, Silva DHS, Bolzani VDS, Gianinni MJSM. The use of standard methodology for determination of antifungal activity of natural products against medical yeasts *Candida* sp and *Cryptococcus* sp. Braz J Microbiol. 2007;38:391-7.

20. Siqueira ER, Ferreira JC, Pedroso RDS, Lavrador MAS, Candido RC. Dermatophyte susceptibilities to antifungal azole agents tested *in vitro* by broth macro and microdilution methods. Rev Inst Med Trop Sao Paulo. 2008;50:1-5.

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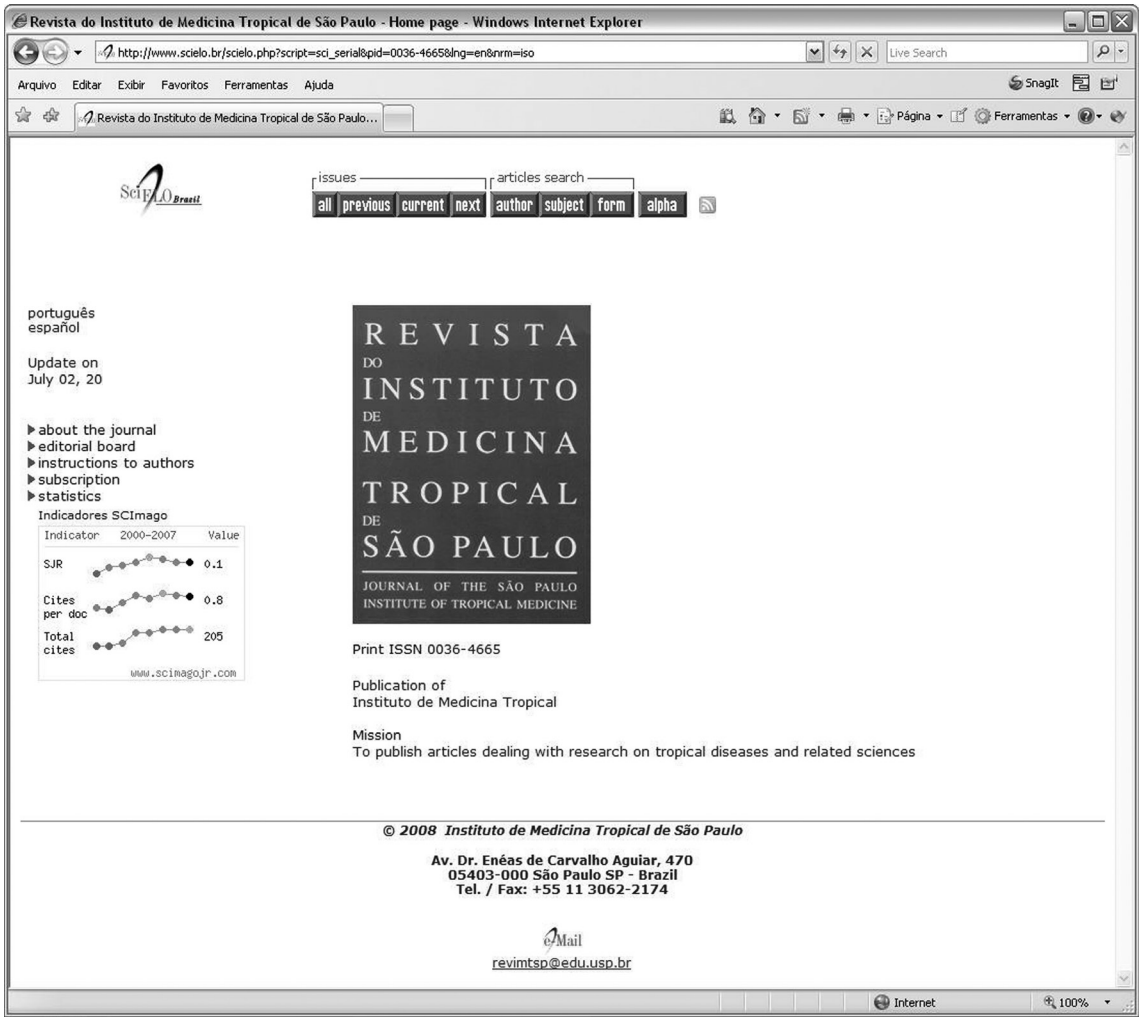

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