sciendo

DOI: 10.2478/fv-2023-0015

FOLIA VETERINARIA, 67, 2: 33—41, 2023

IN VITRO ANTIFUNGAL ACTIVITY OF SELECTED ESSENTIAL OILS AGAINST *TRICHOPHYTON MENTAGROPHYTES*

Lucia Kottferová[1], Eva Čonková[2]
[1]Clinic of Birds, Exotic and Free Living Animals
[2]Department of Pharmacology and Toxicology
University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice
Slovakia

lucia.kottferova@uvlf.sk

ABSTRACT

The *in vitro* activity of *Cinnamomum cassia*, *Melaleuca alternifolia*, and *Cymbopogon citratus* essential oils and antimycotics clotrimazole, fluconazole, and ketokonazole against *T. mentagrophytes* was evaluated by the disc-diffusion method. The essential oils were tested at 5, 10, 20, 30, 50, and 100 % concentration. It was found that *Cymbopogon*, commonly called lemongrass, and cinnamon show stable results independent of the concentration used, whereas the efficacy of tea tree oil, significantly decreases with the decreasing concentration. When comparing the efficacy of antimycotics the largest zone of inhibition was obtained with clotrimazole, while fluconazole proved to be ineffective. We found that cinnamon and lemongrass are more effective than the antimycotics that we have used. Our study confirmed that some essential oils can be used for the treatment of dermatophytoses caused by *Trichophyton mentagrophytes* and some of them are more effective than the commercial drugs.

Key words: antimycotics; *Cinnamomi cassiae aetheroleum; Cymbopogon citratus aetheroleum*; dermatophytoses; *Melaleucae aetheroleum*

INTRODUCTION

Infectious diseases are regarded as a significant problem of health and are a major cause of morbidity and mortality worldwide. Dermatophytosis is quite common among infectious diseases [52].

Fungal infections of the skin are a common global problem. Pathogens responsible for skin mycoses are primarily anthropophilic and zoophilic dermatophytes from the genera *Trichophyton, Microsporum*, and *Epidermophyton*. There appears to be considerable inter- and intracontinental variability in the global incidence of these fungal infections [22, 29].

Clinical signs of trichophytosis are easily observable in humans and other animals. There are circular lesions observed on the skin that may also bind together and form large irregular structures and can have an average of a few centimetres in length [26]. The clinical forms of animal diseases are most often determined by the animal species, the animal´s age, health condition, and virulence process, and may also be determined by individual characteristics of the animal [17, 44]. The presence of inflammation or redness of the infected area and the loss of hair show typical symptoms of the infection [7].

The antifungal drugs that are routinely used the for the

treatment of deep mycoses include polyenes and azoles. An additional drug that sees infrequent use, primarily for life-threatening yeast infections, is flucytosine (5-fluorocytosine). For superficial mycoses, particularly infections caused by fungi, azoles, allylamines, and griseofulvin are most commonly used [23].

Many antifungal medications commonly used in the therapy of fungal diseases have poor efficacy indicating side effects, toxicity, and drug interaction and increasingly also appears the development of resistance to these medications [31, 40]. Treatment of dermatophytosis is quite expensive and time-consuming. Therefore, it is necessary to develop new drugs with improved efficacy and safety or evolve an alternative or combating infections [51]. Natural products have proven to be a source of new alternative active molecules. In many countries, particularly in the developing countries, the plants were primarily used to provide basic medical treatment [32].

The objective of this study was to compare the antifungal activity of essential oils (*Cinnamomi cassiae aetheroleum, Cymbopogon citratus aetheroleum, Melaleucae aetheroleum*) against *T. mentagrophytes* with activity of conventional antimycotics (clotrimazole, fluconazole, ketoconazole) used in clinical practice, using the disc-diffusion method.

MATERIALS AND METHODS

Essential oils

Antifungal activity was tested *in vitro* in three essential oils: cinnamon, tea tree, and lemongrass essential oils (Table 1) (HANUS – Herbal preparations, Nitra, the Slovak Republic) with a concentration of 100 %, 30 %, 20 %, 10 %, and 5 %. While diluting essential oils to the desired concentration, we proceeded exactly as in the preparation of emulsions. Gum Arabic was used as an emulsifier, which represented 30 % of the total concentration of the solution.

Antimycotics

The following commercial antifungal discs were used in the experiment (Biolab Slovakia s. r. o. Komárno, the Slovak Republic): clotrimazole 10 mcg; clotrimazole 50 mcg; fluconazole 25 mcg; ketoconazole 10 mcg.

Tested strain

For testing, we used the reference strain *Trichophyton mentagrophytes* CCM 8377/ATCC 9533 (the Czech Collection of Microorganisms, Brno, the Czech Republic).

Disc-diffusion method

The antifungal activity of essential oils and antimycotics were determined by the disc-diffusion method, according to a standard methodology M44-A2 clinical and laboratory standard institute CLSI for antimicrobial susceptibility testing. The pure culture of the *Trichophyton mentagrophytes* strain was prepared by passaging on Sabouraud dextrose agar with the addition of chloramphenicol. After a 10-day incubation period at room temperature (21–23 °C), a suspension of spores with a sterile saline solution was prepared. The inoculum density was adjusted using a densitometer (Denzi-Lameter, Pliva LaChema a. s., Brno, the Czech Republic) to the 0.5 McFarland-scale equivalent (i. e. $1 \times 10^6 – 5 \times 10^6$ CFU.ml^{-1}). Subsequently, the inoculum was applied with a sterile swab onto the surface of the nutrient medium twice in three directions, with 15 minutes between the two measurements. The discs

Table 1. The essential oils tested *in vitro* against *T. mentagrophytes*

Type of essential oil type	Latin name of the essential oil	Latin name of the mother plant	Latin name of the family
Cinnamon	*Cinnamomi cassiae aetheroleum*	*Cinnamomum cassia*	Lauraceae
Tea tree	*Melaleucae aetheroleum*	*Melaleuca alternifolia*	Myrtaceae
Lemongrass	*Cymbopogon citratus aetheroleum*	*Cymbopogon citratus*	Poaceae

associated with antimycotics, or essential oils at the appropriate concentration were transferred onto the prepared medium. The plates were incubated at a laboratory temperature for 10 days and then the results were counted. A zone of inhibition was created as the criterion for determining sensitivity, where the size reached at least twice the size of the disc. The experiment was repeated 3 times. When testing azole antimycotics, the sensitivity was evaluated based on the criteria in the manufacturer´s instructions.

Statistical analysis

The statistical analysis was performed by regression analysis. To test the differences among medium values of multiple files, the method ANOVA and Tukey's multiple comparison method were used. All statistical hypotheses were tested at a significance level of $\alpha \leq 0.05$.

RESULTS

The average values of the zones of inhibition against *T. mentagrophytes* are shown in Table 2. Lemongrass and cinnamon showed higher average values than the other tested substances. The median of the two essential oils reached even the highest possible value of 90 mm (i. e., the diameter of the Petri dish). We observed a decrease in the medium value of the lemongrass against cinnamon, which was caused by low values at 5 % concentration. Tea tree, fluconazole, and ketoconazole showed considerably lower values than the other tested substances. All of the substances, except the tea tree oil, resulted in very low values of the coefficient of variation, so their effects could be considered as stable.

Conventional antimycotics showed a stable effect since a low coefficient of variation was detected. Essential oils, cinnamon, and lemongrass reached the highest efficacy from the concentration of 10 %. The dose-dependent efficacy of essential oils concentration was verified by regression analysis separately for each essential oil (Tables 3, 4).

From Table 3 it is evident that the efficacy level of lemongrass did not depend on the concentration. The p-value of the linear coefficient was greater than 0.05 while that of the constant coefficient was very close to zero, thus the constant coefficient was significant.

From Table 4 it is evident that the efficacy value of the tea tree depends on the concentration. The p-value of the linear and constant coefficients was less than 0.05, and both coefficients were significant.

There was no statistical testing with the cinnamon, as the results of the measured values were equal to 90. Thus, a constant dependency was evident.

A one-way analysis by Tukey's method was employed for the multiple comparison efficacies of each concentration of essential oils for the concentrations ranging from 5 to 20 % (Table 5), 30 % (Table 6), and 100 %.

At a 100 % oil concentration it was impossible to find any differences among the substances since almost at every measurement the diameter of the inhibition zone reached 90 mm.

Table 2. The average values (mm) of the zones of inhibition against *T. mentagrophytes*

	Medium value	Median	Coefficient of variation	Susceptible/ resistant (S/R)
Lemongrass	78.53	90	0.3	S
Cinnamon	90	90	0	S
Tea tree	24.66	0	1.48	S
Clotrimazole 10	76.66	80	0.07	S
Clotrimazole 50	76.66	80	0.07	S
Fluconazole	0	0	0	R
Ketokonazole	28.66	29	0.08	S

Table 3. Linear regression for lemongrass

	Coefficients	Medium value error	t Stat	P-value
Constant coefficient	69.13	8.53	8.1	$3.302e^{-6}$*
Linear coefficient	0.30	0.20	1.48	0.163609

* – P ≤ 0.05

Table 4. Linear regression for tea tree

	Coefficients	Medium value error	t Stat	P-value
Constant coefficient	–7.93	3.82	–2.07	0.0096019*
Linear coefficient	0.99	0.09	10.78	$0.01581e^{-7}$*

* – P ≤ 0.05

Table 5. Analysis by Tukey´s method of 5–20 % concentration of essential oils

	Diameter difference [mm]	Lower estimate	Upper estimate	P-value
Lemongrass – tea tree	69.77	50.04	89.51	0*
Cinnamon – tea tree	88.88	69.15	108.62	0*
Cinnamon – lemongrass	19.11	–0.62	38.84	0.05

P ≤ 0.05

Table 6. Analysis by Tukey´s method for 30 % concentration of essential oils

	Diameter difference	Lower estimate	Upper estimate	P-value
Lemongrass – tea tree	60	31.07	88.92	0.0017169*
Cinnamon – tea tree	60	31.07	88.92	0.0017169*
Cinnamon – lemongrass	–1.421085E-14	–28.92	28.92	1.00

* – P ≤ 0.05

DISCUSSION

In vitro antimicrobial activity of essential oils of cinnamon was published in many studies [1, 5, 11, 12, 15, 18, 19, 21, 30, 35, 39, 42, 43, 45]. The antifungal effects of essential oil from *Cinnamomum longepaniculatum* leaves were studied with special reference to minimal inhibitory concentration (MIC), minimal fungicidal concentration (MFC), time-kill studies, and the mechanism of inhibition at an ultrastructural level against *Trichophyton mentagrophytes*, *Microsporum canis* and *Trichophyton gypseum* [47]. Our results also confirmed the antifungal effect of *Cinnamomum cassia*. In our experiment, the efficacy of *Cinnamomum cassia* against *Trichophyton mentagrophytes* did not depend on concentration of the essential oil. The average values of the zones of inhibition were determined at all tested concentrations at 90 mm as the maximum possible value.

In vitro and *in vivo* studies conducted in different countries demonstrated numerous beneficial medicinal effects

of *Cinnamomum zeylanicum* [41] and its anti-microbial activity [25]. T a o et al. confirmed that essential oil from *Cinnamomum longepaniculatum* showed a significant antifungal activity *in vivo*, weakening the virulence of three dermatophytes and killing fungi by destroying the cell membrane and organelles [47].

The aim of the study by C i s a r o v á et al. [9] was to assess the antifungal and anti-toxinogenic activity of 15 essential oils against three fungi of the genus *Aspergillus*. All essential oils exhibited activity against the tested strains of fungi.

G o z u b u y u k et al. [20] investigated the antifungal activity of *L. inermis* (henna) against six different dermatophyte species: *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Trichophyton tonsurans*, *Epidermophyton floccosum*, and *Trichophyton violaceum*. The antifungal activity of *L. inermis* was determined by agar diffusion method and henna was used in paste form. Henna paste showed high antifungal activity against all dermatophytes species.

The antifungal potential of tea tree was investigated *in vitro* against *Trichophyton rubrum* and *T. mentagrophytes* var. interdigitale, the most prevalent causes of tinea and onychomycosis infection in humans. There was observed a clear antifungal action on these organisms grown in culture. B e n g e r et al. [3] and C a s s e l l a et al. [8] detected the activity of *Melaleuca alternifolia* against the dermatophyte *Trichophyton rubrum*. The studies also confirmed that the oil was a potent antifungal agent and that the inverse relationship between essential oil concentration and fungal growth was not influenced by random variability. Our study showed that the efficacy of this essential oil against *T. Mentagrophyte*, was maximal at 100% concentration (inhibition zone of 90 mm), but that the effectiveness was significantly decreased at a concentration of 30%. The tea tree showed an inhibition zone diameter of 0 mm at concentrations ranging from 5 to 20% at which it can be considered ineffective.

C o n c h a et al. [10] determined the activity of tea tree oil against 58 clinical isolates. This *in vitro* study indicated that tea tree oil may be useful in the treatment of yeast and fungal mucosal and skin infections.

Antimicrobial activities of methanol leaf extract of *Leucas* sp. were studied by B a b u et al. [2]. Antifungal susceptibilities of clinically isolated dermatophytes to methanol extracts of *Leucas aspera* and *Leucas zeylanica* leaves were investigated using the agar well diffusion method. The results obtained showed that all the extracts expressed remarkable antifungal activity. The maximum inhibition zone was recorded with *Penicillium* sp. while the minimum inhibition zone was recorded for *Candida tropicalis*. These results confirmed potential anti-dermatophytic activity of *Leucas* spp.

The antimycobacterial activity of aerial parts and essential oils of four *Salvia* species against *Mycobacterium tuberculosis* strains was analysed and the antifungal activity against dermatophytes *Microsporum gypseum* and *Trichophyton mentagrophytes* and also *Candida* species was compared with by broth microdilution method. The essential oils showed high antimycobacterial and antifungal activity [46].

The *in vitro* activity of twenty chemically defined essential oils against *Microsporum canis, Trichophyton mentagrophytes*, *T. erinacei, T. terrestre* and *Microsporum gypseum Microsporum canis*, *Trichophyton mentagrophytes, T. erinacei, T. terrestre* and *Microsporum gypseum* was assayed. More effective essential oils were *T. serpyllum, O. vulgare*, and *L. cubeba. F. vulgare* showed moderate efficacy against geophilic species such as *M. gypseum* and *T. terrestre* [34].

D e s a m et al. [13] studied the antifungal activity of mentha and peppermint essential oils. They showed a significant antifungal activity against *Alternaria alternaria, Fusarium tabacinum, Penicillum* spp. *Fusarium oxyporum* and *Aspergillus fumigates*.

The study conducted with south Indian medicinal plants involved their efficacy against three clinical fungal isolates *Trichophyton mentagrophytes, Epidermophyton floccosum,* and *Candida albicans*, using the agar well diffusion method. The extract of twenty plants used in this study acted as a good source of antibiotics against various fungal pathogens tested and exhibited a broad spectrum of antifungal activity [49].

Several authors investigated the antibacterial properties of *Cymbopogon citratus* [24, 36, 37]. K h a n and A h m a d [28] evaluated *in vitro* the antifungal activity of *Cymbopogon citratus* oil against azole-resistant strains of *Aspergillus* spp. and *Trichophyton* spp. They observed that *C. citratus* exhibited promising antifungal activity (zone of inhibition from 24.66 to 42.00 mm) and the killing potency against *Aspergillus fumigatus* and *Trichophyton rubrum*. Our results showed that the efficacy of *Cym-*

bopogon citratus did not depend on the concentration. It showed the highest possible efficacy against *T. mentagrophytes* at concentrations ranging from 10 to 100 %. The efficacy decreased (32.6 mm) at 5 % concentration.

B i a s i - G a r b i n et al [4] evaluated the *in vitro* antidermatophytic activity of 23 crude extracts from nine plant species found in Brazil. The extracts were tested by broth microdilution assay against the reference strains of *T. rubrum* and *T. mentagrophytes* and 33 clinical isolates of dermatophytes. All plants showed a fungicidal effect against both fungal species. Selected extracts of *Eugenia uniflora, Libidibia ferrea*, and *Persea americana* also exhibited a fungicidal effect against all clinical isolates of *T. rubrum* and *T. mentagrophytes* complex. This is the first report of the antifungal activity of *Schinus terebinthifolius, Piptadenia colubrina, Parapiptadeniarigida, Mimosa ophthalmocentra*, and *Persea americana* against both dermatophyte species.

O r c h a r d et al. reported high susceptibility of fungal pathogens, such as *Trichophyton mentagrophytes*, to combinations of essential oils. The potential combinations containing *Cinnamomum verum* or *Santalum austrocaledonicum* were studied in a clinical settings with the goal to decrease the need for systemic or prolonged antifungal treatments that may result in a treatment failure or resistance [38]. Essential oil mixtures may provide a better outcome than monotherapy in terms of side effects and toxicity, as well as reducing the emergence of resistant strains. Based on their antifungal and anti-inflammatory properties, these combinatorial strategies may be useful in supplementing conventional therapy by alleviating symptoms, promoting healing, and preventing spread of dermatophytosis [48].

Overall, we can summarize our findings that lemongrass and cinnamon have significantly better efficacy than tea tree, except for the concentration of 100 %, and their effect did not dependent on the tested concentration. The efficacy was the highest possible and the zone inhibition diameter was 90 mm. The statistical comparison shows that there are significant differences between lemongrass and cinnamon compared to tea tree. Z e n g, C h e n and L i a n g [53] studied the antifungal effects of fennel seed essential oil against *Trichophyton rubrum, Trichophyton tonsurans, Microsporum gypseum* and *Trichophyton mentagrophytes*. Fennel seed essential oil was investigated from various aspects, such as MIC and minimum fungicidal concentration, mycelia growth, spore germination, and biomass. The results indicated that this oil showed better antifungal activities than the commonly used antifungal agents fluconazole and amphotericin B.

Our experiments showed that the efficacy was less than 90 with each conventionally produced antifungal agent tested in our study and the essential oils such as cinnamon and lemongrass were more effective as antimycotics. D i o g o et al. [14] investigated the activity of different concentrations of terbinafine against *T. mentagrophytes* using the disc-diffusion method. The diameter of the inhibition zone was 40 mm and did not depend on the concentration of terbinafine. Comparing the effectiveness of conventional antimycotics, the largest inhibition zone was observed with clotrimazole (80 mm), but fluconazole proved ineffective against *T. mentagrophytes* and ketoconazole showed an inhibition zone of 29 mm in our experiment. The effectiveness of clotrimazole did not depend on concentration. K e y v a n et al. [27] conducted *in vitro* activity testing of six antifungal drugs against clinically important dermatophytes and reported that fluconazole exhibited the poorest activity while clotrimazole and terbinafine were the most effective antifungal drugs. E s t e b a n et al. [16] also tested by disc-diffusion method the antifungal susceptibility of six kinds of dermatophytes including *T. mentagrophytes* and confirmed the high efficiency of clotrimazole.

Despite promising results of recent studies [6, 33, 50], we agree with their authors that the potential toxicity of these products must be considered because EO have been shown to cause skin allergy and irritation. As a result, it is critical not only to investigate the effectiveness of essential oils, but also pay attention to their safety (harmlessness) as well as the potential associated risks.

CONCLUSIONS

Essential oils have long been known to be in a centre of attention for their antimicrobial effects because of their potential role as an alternative to antibiotics and antimycotics for therapeutic purposes. The *in vitro* experiment, based on descriptive statistics, indicated that lemongrass and cinnamon essential oils were effective against *T. mentagrophytes*, while the effectiveness of tea tree decreased significantly depending on concentration. When compar-

ing the efficacy of commercial antimycotics, the greatest commercial inhibition zone was observed with clotrimazole, and fluconazole has proved ineffective.

The disc-diffusion method used in this study is a simple and valuable method for the evaluation of the antifungal susceptibility of dermatophytes in *in vitro* experiments.

The antifungal effects of essential oils could be a very promising solution that can overcome the therapeutic shortcomings of antimycotic medication. More experiments are needed to examine the properties of these oils in order to devise effective and nonaggressive therapies for the treatment of dermatophytosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The study was supported by the KEGA 009UVLF-4/2022.

REFERENCES

1. **Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M., 1998:** Antifungal activities of *Origanum vulgare* subsp. *hirtum, Mentha spicata, Lavandula angustifolia,* and *Salvia fruticosa* essential oils against human pathogenic fungi. *J. Agric. Food Chem.*, 46, 1739–1745.
2. **Babu, A., Mohamed, M. S. N., Jaikumar, K., Anand, D., Saravanan, P., 2016:** *In vitro* antifungal activity of leaf extracts of *Leucas aspera* and *Leucas zeylanica*. *IJPSR*, 7, 752. DOI: 10.13040/IJPSR.0975-8232.7(2).752-56.
3. **Benger, S., Townsend, P., Ashford, R. L., Lambert, P., 2004:** An *in vitro* study to determine the minimum inhibitory concentration of *Melaleuca alternifolia* against the dermatophyte *Trichophyton rubrum*. *The Foot*, 14, 86–91.
4. **Biasi-Garbin, R. P., Demitto, F. D. O., Amaral, R. C. R. D., Ferreira, M. R. A., Soares, L. A. L., Svidzinski, T. I. E., Yamada-Ogatta, S. F., 2016:** Antifungal potential of plant species from Brazilian Caatinga against dermatophytes. *Revista do Instituto de Medicina Tropical de São Paulo*, 58. DOI: 10.1590/S1678-9946201658018.
5. **Biondi, D., Cianci, P., Geraci, C., Ruberto, G., Piattelli, M., 1993:** Antimicrobial activity and chemical composition of essential oils from Sicilian aromatic plants. *Flavour Fragr. J.*, 8, 331–337.
6. **Bogavac, M., Karaman, M., Janjušević, L., Sudji, J., Radovanović, B., Novaković, Z., et al., 2015:** Alternative treatment of vaginal infections – *in vitro* antimicrobial and toxic effects of *Coriandrum sativum* L. and *Thymus vulgaris* L. essential oils. *J. Appl. Microbiol.*, 119, 3, 697–710. DOI: 10.1111/jam.12883.
7. **Carrillo-Munoz, A. J., Giusiano, G., Ezkurra, P. A., Quindos, G., 2006:** Antifungal agents: Mode of action in yeast cells. *Rev. Esp. Quimioter*, 19, 130–139.
8. **Cassella, S., Cassella, J. P., Smith, I., 2002:** Synergistic antifungal activity of tea tree (*Melaleuca alternifolia*) and lavender (*Lavandula angustifolia*) essential oils against dermatophyte infection. *Int. J. Aromath.*, 12, 2–15.
9. **Císarová, M., Tančinová, D., Medo, J., Kačániová, M., 2016:** The *in vitro* effect of selected essential oils on the growth and mycotoxin production of *Aspergillus* species. *J. Environ. Health*, Part B, 51, 668–674. DOI: 10.1080/03601234.2016.1191887.
10. **Concha, J. M., Moore, L. S., Holloway, W. J., 1998:** Antifungal activity of *Melaleuca alternifolia* (tea-tree) oil against various pathogenic organisms. *J. Am. Podiatr. Med. Assoc.*, 88, 489–492.
11. **Cosentino, S., Tuberoso, C. I. G., Pisano, B., Satta, M., Mascia, V., Arzedi, E., Palmas, F., 1999:** *In vitro* antimicrobial activity and chemical composition of *Sardinian thymus* essential oils. *Lett. Appl. Microbiol.*, 29, 130–135.
12. **Deans, S. G., Ritchie, G., 1987:** Antibacterial properties of plant essential oils. *Int. J. Food Microbiol.*, 5, 165–180.
13. **Desam, N. R., Al-Rajab, A. J., Sharma, M., Mylabathula, M. M., Gowkanapalli, R. R., Albratty, M., 2017:** Chemical constituents, *in vitro* antibacterial and antifungal activity of *Mentha Piperita* L. (peppermint) essential oils. *J. King Saud Univ. Sci*. DOI: 10.1016/j.jksus.2017.07.013.
14. **Diogo, H. C., Melhem, M., Sarpieri, A., Pires, M. C., 2010:** Avaliação do método de disco-difusão para determinação da eficácia da terbinafina *in vitro* em agentes de micoses superficiais e subcutâneas. *An. Bras. Dermatol.*, 85, 324–330. DOI: 10.1590/S0365-05962010000300005.
15. **Dorman, H. J. D., Deans, S. G., 2000:** Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J. Appl. Microbiol.*, 88, 308–316.
16. **Esteban, A., Abarca, M. L., Cabanes, F. J., 2005:** Compar-

ison of disk diffusion method and broth microdilution method for antifungal susceptibility testing of dermatophytes. *Med. Mycol.*, 43, 61–66. DOI: 10.1080/13693780410001711972.

17. **Fabikova, R., Kuttova, M., Huml, O., Cada, F., Stichova, S., 1995:** Evaluation of bacterial, mycological and parasitary findings from dermatological examination of dogs and cats in 1994 (In Czech). *Veterinarstvi*, 45, 447–451.
18. **Farag, R. S., Daw, Z. Y., Abo-Raya, S. H., 1989:** Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *J. Food Sci.*, 54, 74–76.
19. **Ferhout, H., Bohatier, J., Guillot, J., Chalchat, J. C., 1999:** Antifungal activity of selected essential oils, cinnamaldehyde and carvacrol against *Malassezia furfur* and *Candida albicans. J. Essent. Oil Res.*, 11, 119–129.
20. **Gozubuyuk, G. S., Aktas, E., Yigit, N., 2014:** An ancient plant *Lawsonia inermis* (henna): Determination of *in vitro* antifungal activity against dermatophytes species. *J. Mycol. Med.*, 24, 313–318. DOI: 10.1016/j.mycmed.2014.07.002.
21. **Hammer, K. A., Carson, C. F., Riley, T. V., 1999:** Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.*, 86, 985–990.
22. **Havlickova, B., Czaika, V. A., Friedrich, M., 2008:** Epidemiological trends in skin mycoses worldwide. *Mycoses*, 51, 2–15. DOI: 10.1111/j.1439-0507.2008.01606.x.
23. **Hector, R. F., 2005:** An overview of antifungal drugs and their use for treatment of deep and superficial mycoses in animals. *Clin. Tech. Small Anim. Pract.*, 20, 240–249. DOI: 10.1053/j.ctsap.2005.07.005.
24. **Chandrashekar, K. S., Joshi, A. B., 2006:** Antifungal efficacy of essential oil of *Cymbopogon* species against some dermatophytes. *Ind. Perf.*, 50, 54.
25. **Jayaprakasha, G. K., Rao, L. J. M., 2011:** Chemistry, biogenesis, and biological activities of *Cinnamomum zeylanicum. Crit. Rev. Food Sci. Nutr.*, 51, 547–562. DOI: 10.1080/10408391003699550.
26. **Kaplan, W., Gump, R. H., 1958:** Ringworm in a dog caused by *Trichophyton rubrum. Vet. Med.*, 53, 139–142.
27. **Keyvan, P., Leila, B., Zahra, R., Manuchehr, S., Kamiar, Z., 2009:** *In vitro* activity of six antifungal drugs against clinically important dermatophytes. *Jundishapur J. Microbiol.*, 4, 158–163.
28. **Khan, M. S. A., Ahmad, I., 2013:** *In vitro* antifungal activity of oil of *Cymbopogon citratus* and citral alone and in combination with fluconazole against azole-resistant strains of *Aspergillus fumigatus* and *Trichophyton rubrum. Phcog. Commn.*, 3, 29. DOI: 10.5530/pc.2013.3.6.
29. **Lakshmipathy, D. T., Kannabiran, K., 2010:** Review on dermatomycosis: Pathogenesis and treatment. *Nature*, 2, 726. DOI: 10.4236/ns.2010.27090.
30. **Manou, I., Bouillard, L., Devleeschouwer, M. J., Barel, A. O., 1998:** Evaluation of the preservative properties of *Thymus vulgaris* essential oil in topically applied formulations under a challenge test. *J. Appl. Microbiol.*, 84, 368–376.
31. **Martinez-Rossi, N. M., Peres, N. T., Rossi, A., 2008:** Antifungal resistance mechanisms in dermatophytes. *Mycopathologia*, 166, 369–383. DOI: 10.1007/s11046-008-9110-7.
32. **Meléndez, P. A., Capriles, V. A., 2006:** Antibacterial properties of tropical plants from Puerto Rico. *Phytomedicine*, 13, 272–276. DOI: 10.1016/j.phymed.2004.11.009.
33. **Mutlu-Ingok, A., Devecioglu, D., Dikmetas, D. N., Karbancioglu-Guler, F., Capanoglu, E., 2020:** Antibacterial, antifungal, antimycotoxigenic, and antioxidant activities of essential oils: An updated review (Open Access), *Molecules*, 25, 20, Art. No. 4711. DOI: 10.3390/molecules25204711.
34. **Nardoni, S., Giovanelli, S., Pistelli, L., Mugnaini, L., Profili, G., Pisseri, F., Mancianti, F., 2015:** *In vitro* activity of twenty commercially available, plant-derived essential oils against selected dermatophyte species. *Nat. Prod. Commun.*, 10. DOI: 10.1177/1934578X1501000840.
35. **Nelson, R. R., 1997:** *In vitro* activities of five plant essential oils against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium. J. Antimicrob. Chemother.*, 40, 305–306.
36. **Nwachukwu, I. N., Allison, L. N., Chinakwe, E. C., Nwadiaro, P., 2008:** Studies on the effects *Cymbopogon citratus, Ceiba pentandra* and *Loranthus bengwelensis* extracts on species of dermatophytes. *J. Am. Sci.*, 4, 58–67.
37. **Onawunmi, G. O., Yisak, W. A., Ogunlana, E. O., 1984:** Antibacterial constituents in the essential oil of *Cymbopogon citratus* (DC.). *Stapf. J. Ethnopharm.*, 12, 279–286.
38. **Orchard, A., van Vuuren, S. F., Viljoen, A. M., 2019:** Commercial essential oil combinations against topical fungal pathogens. *Nat. Prod. Comm.*, 14. DOI: 10.1177/1934578X1901400139.
39. **Paster, N., Juven, B. J., Shaaya, E., Menasherov, M., Nitzan, R., Weisslowicz, H., Ravid, U., 1990:** Inhibitory effect of oregano and thyme essential oils on moulds and foodborne bacteria. *Lett. Appl. Microbiol.*, 11, 33–37.
40. **Pfaller, M. A., Boyken, L., Hollis, R. J., Kroeger, J., Messer, S. A., Tendolkar, S., Diekema, D. J., 2008:** *In vitro* sus-

ceptibility of invasive isolates of *Candida* spp. to anidulafungin, caspofungin, and micafungin: Six years of global surveillance. *J. Clin. Microbiol.*, 46, 150–156. DOI: 10.1128/JCM.01901-07.

41. **Ranasinghe, P., Pigera, S., Premakumara, G. S., Galappaththy, P., Constantine, G. R., Katulanda, P., 2013:** Medicinal properties of 'true' cinnamon (*Cinnamomum zeylanicum*): A systematic review. *BMC Complement. Altern. Med.*, 13, 275. DOI: 10.1186/1472-6882-13-275.
42. **Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T., Arsenakis, M., 1996:** Antimicrobial and cytotoxic activities of *Origanum* essential oils. *J. Agric. Food Chem.*, 44, 1202–1205.
43. **Smith-Palmer, A., Stewart, J., Fyfe, L., 1998:** Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Lett. Appl. Microbiol.*, 26, 118–122.
44. **Stewart, L. J., 1990:** Newly reported skin disease syndromes in the dog. *Veterinary Clinics of North America: Small Animal Practice*, 20, 1603–1613.
45. **Stiles, J. C., Sparks, W., Ronzio, R. A., 1995:** The inhibition of *Candida albicans* by oregano. *J. Appl. Nutr.*, 47, 96–102.
46. **Tan, N., Satana, D., Sen, B., Tan, E., Altan, H. B., Demirci, B., Uzun, M., 2016:** Antimycobacterial and antifungal activities of selected four salvia species. *Rec. Nat. Prod.*, 10, 593.
47. **Tao, C., Wei, Q., Yin, Z. Q., Zhou, L. J., Jia, R. Y., Xu, J., Deng, Y. X., 2013:** Antifungal activity of essential oil from *Cinnamomum longepaniculatum* leaves against three dermatophytes *in vitro*. *Afr. J. Pharm.*, 7, 1148–1152. DOI: 10.5897/AJPP11.013.
48. **Trifan, A., Luca, S. V., Bostănaru, A. C., Brebu, M., Jităreanu, A., Cristina, R. T., Mareş, M., 2021:** Apiaceae essential oils: boosters of terbinafine activity against dermatophytes and potent anti-inflammatory effectors. *Plants*, 10, 11, 2378. DOI: 10.3390/plants10112378.
49. **Uddandapu, P. K., Naidu, K. C., Rao, Y. V., 2016:** *In vitro* anti-fungal activity of few medicinal plants of Visakhapatnam region against clinically isolated dermatophytes. *Int. J. Bioassays*, 5, 4573–4578. DOI: 10.21746/ijbio.2016.05.008.
50. **Villar Rodríguez, J., Pérez-Pico, A. M., Mingorance-Álvarez, E., Mayordomo Acevedo, R., 2022:** Meta-analysis of the antifungal activities of three essential oils as alternative therapies in dermatophytosis infections. *J. Appl. Microbiol.*, 133, 2, 241–253. DOI: 10.1111/jam.15539.
51. **Wang, J., Li, J., Cao, J., Jiang, W., 2010:** Antifungal activities of neem (*Azadirachta indica*) seed kernel extracts on postharvest diseases in fruits. *Afr. J. Microbiol. Res.*, 4, 1100–1104.
52. **World Health Organization, 2004:** *The World Health Report Changing History. Statistical Annex. Deaths by Cause, Sex and Mortality Stratum in WHO Regions Estimates for 2002.* Geneva, Switzerland, WHO, 120–122.
53. **Zeng, H., Chen, X., Liang, J., 2015:** *In vitro* antifungal activity and mechanism of essential oil from fennel (*Foeniculum vulgare* L.) on dermatophyte species. *J. Med. Microbiol.*, 64, 93–103. DOI: 10.1099/jmm.0.077768-0.

Received April 5, 2023
Accepted May 15, 2023