Corymbia citriodora Essential Oil Antimicrobial Activity *in vitro*

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Abstract
Medium to large evergreen *Corymbia citriodora* (*Eucalyptus citriodora*) trees have smooth, powdery bark that is light grey, cream, or pink in color. It is frequently grown as an ornamental tree and is widely cultivated in Australia for its timber and essential oil. Herbal remedies are made from its foliage. It produces an essential oil that can be used to treat a variety of ailments, including clogged nasal passageways, cuts wounds, sore throats, and skin infections. The aim of the study was to determine the antimicrobial activity of *Corymbia citriodora* essential oil (CCEO) against ten microorganisms *Candida albicans* CCM 8186, *C. glabrata* CCM 8270, *C. krusei* CCM 8271, *C. tropicalis* CCM 8223, *Staphylococcus aureus* subsp. *aureus* CCM 4223, *Micrococcus luteus* CCM 732, *Listeria monocytogenes* CCM 4699, *Escherichia coli* CCM 3954, *Pseudomonas putida* CCM 1977, and *Enterobacter aerogenes* CCM 2531. The antimicrobial activity of CCEOs was evaluated using the disc diffusion method. The best antibacterial activity of CCEO against yeasts was found against *C. tropicalis* (17.33±0.58 mm). CCEO was most effective against Gram-positive bacteria *M. luteus* (9.33±0.58 mm) and Gram-negative bacteria *E. coli* (12.67±0.58 mm). In conclusion, *Candida* species were most sensitive against *Corymbia citriodora* essential oil.

Keywords: *Corymbia citidora* essential oil, antimicrobial activity, Gram-positive bacteria, Gram-negative bacteria, yeasts

1. Introduction

There are about 900 species and subspecies that are contained in the Eucalyptus genus [1]. The genus Eucalyptus is native to Australia, but due to its easy cultivation, it is already widespread all over the world [2]. One of the main reasons for worldwide cultivation of the Eucalyptus is to obtain pulp, gum, wood, and essential oils (EO).
Corymbia citriodora EO (CCEO) from the genus of Eucalyptus is mainly extracted from the leaves, and is often used in medicine, perfumery, and food industries, mainly due to its biological activities including antimicrobial, antiseptic, antioxidant, chemotherapeutic, respiratory, and gastrointestinal disorders, wound healing, and insecticidal/repellent, herbicidal, acaricidal, nematocidal, and perfume, soap making and grease remover [3].
Since EOs have shown different levels of biological effects, they may one day be employed alone, or in a combination with other compounds to create new medications. Yet, the various bioactivities that need to be assessed are determined by their chemical variability based on species, varieties, or geographical origin [4].
Previous research showed that CCEOs and their major constituents exhibit inhibitory and toxic effects against a wide range of microorganisms including bacteria, fungi, and yeasts [5]. The antimicrobial activity of CCEO can be attributed to the presence of compounds such as 1,8-cineole, α-pinene, β-pinene and limonene [6].
Our aim in this context is to investigate the potential use of *Corymbia citriodora* leaf essential oil as a

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natural antimicrobial agent. In that regard we have examined its effect on selected candida, Gram-positive bacteria, and Gram-negative bacteria.

2. Materials and methods

Essential oil

Corymbia citriodora essential oil (CCEO) was purchased from Hanus Company (Nitra, Slovakia). EO was produced by steam distillation of leaves, and the major components were citronellal, citronellol, citronellyl acetate, limonene, 1,8-cineole, ß-pinene, α-pinene, ß-caryophyllene, linalool, eugenol. The country of origin of essential oil is from Brazil.

Microorganisms

Four yeasts *Candida albicans* CCM 8186, *C. glabrata* CCM 8270, *C. krusei* CCM 8271, *C. tropicalis* CCM 8223, three Garam-positive bacteria *Staphylococcus aureus* subsp. *aureus* CCM 4223, *Micrococcus luteus* CCM 732, *Listeria monocytogenes* CCM 4699, three Gram-negative bacteria *Escherichia coli* CCM 3954, *Pseudomonas putida* CCM 1977, and *Enterobacter aerogenes* CCM 2531 were obtained from Czech Collection of Microorganisms (CCM, Brno, Czech Republic).

Disc diffusion method

The antimicrobial activity of CCEO was initially assessed using the disc diffusion method. CCEO at 100 %, 50 %, 25 %, and 12.5 % concentration was used. CCEO was diluted with 1 % dimethyl sulfoxide (DMSO). The yeast inoculum was grown for 24 hours on Sabouraud dextrose agar (SDA, Oxoid, Basingstoke, UK), while the bacteria were grown for 24 hours on Tryptone soya agar (TSA, Oxoid, Basingstoke, UK). Inoculums of bacteria and yeast were maintained at 37 °C and 25 °C. Using purified water, the microbial culture was brought to an optical density of 0.5 McFarland standard (1.5 10^8 CFU/mL). On either Sabouraud dextrose agar (SDA, Oxoid, Basingstoke, UK) for yeasts or Mueller-Hinton agar (MHA, Oxoid, Basingstoke, UK) for bacteria were 100 microliters of cultures spread. After that, sterile 6 mm discs saturated with 10 L of CCEO were placed on the microbial suspension, and plates were then incubated for 24 hours at 37 °C for bacteria and 25 °C for yeasts. Three sides of the filter's edge were measured for inhibition zones, and the findings were interpreted as follows: inhibition zones larger than 10 mm had very strong antimicrobial activity; 10-5 mm had moderate activity; and 5-1 mm had weak activity. Meropenem (Oxoid, Basingstoke, UK) and fluconazole (Oxoid, Basingstoke, UK) were the antibiotics used as controls for bacteria and yeasts, respectively. All measurements were performed in triplicate.

Statistical analyses

Triplicates of each test and analysis were performed. The Excel 2020 program from Microsoft was used to compute the mean and standard deviation (SD).

3. Results and discussion

Antifungal activity

The antimicrobial activity of CCEO against *C. albicans* showed Figure 1. The best antimicrobial activity was found with 100 % concentration (16.33±0.58 mm). Following agar disc diffusion and broth dilution assay procedures, the antimicrobial activity of *Corymbia citriodora* essential oil against pathogenic fungi, bacteria, and drug-resistant mutants of *Candida albicans*, *Escherichia coli*, and *Mycobacterium smegmatis* was assessed both qualitatively, and quantitatively. *Trichophyton rubrum*, *Histoplasma capsulatum*, *Candida albicans* (MTCC), and *Cryptococcus neoformans* were found to be the most susceptible to the CCEO. Additionally, it demonstrated action against drug-resistant mutants of *C. albicans* and *E. coli* as well as Gram-positive bacteria as opposed to Gram-negative bacteria. The results of our pilot research indicate that identifying and isolating the active phytoceutical(s) from the CCEO may yield a useful antimicrobial agent for combating fungi and infections with drug resistance [7].

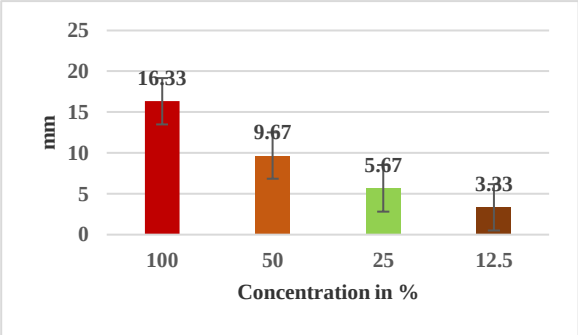

Figure 1. Antimicrobial activity of CCEO against *Candida albicans*

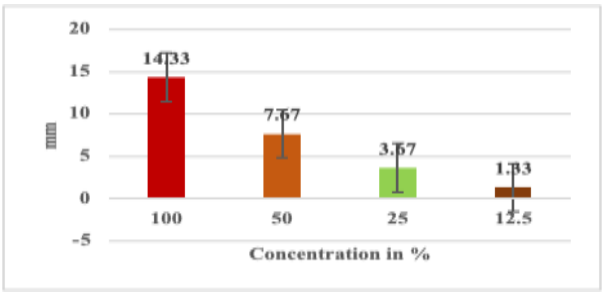

Figure 2. Antimicrobial activity of CCEO against *Candida glabrata*

The antimicrobial activity of CCEO against *C. glabrata* showed Figure 2. The best antimicrobial activity was found with 100 % concentration (14.33±0.58 mm). With the emergence of resistance against the currently available antimicrobial agents, another study was conducted with the aim of evaluating the antimicrobial property of eucalyptus essential oil, and testing its efficacy against drug-resistant mutants of *C. albicans*, *E. coli*, and *M. smegmatis* [8–17].

The antimicrobial activity of CCEO against *C. glabrata* showed Figure 3. The best antimicrobial activity was found with 100 % concentration (15.67±0.58 mm).

Citronellal alone was found to be more effective than eucalyptus oil, according to Ramezani et al. [18], who investigated the fungicidal effect of citronellal against *Rhizoctonia solani*, and *Helminthosporium oryzae*. Citronellol's inhibition rates against *Phytophthora cactorum* were close to 100%, according to research by Lee et al. [19] on the antifungal action. While, no effort was made to concentrate on the main compounds citronellal, and citronellol's antidermatophytic properties.

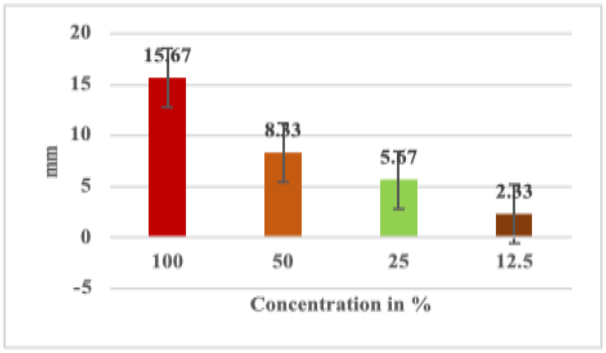

Figure 3. Antimicrobial activity of CCEO against *Candida krusei*

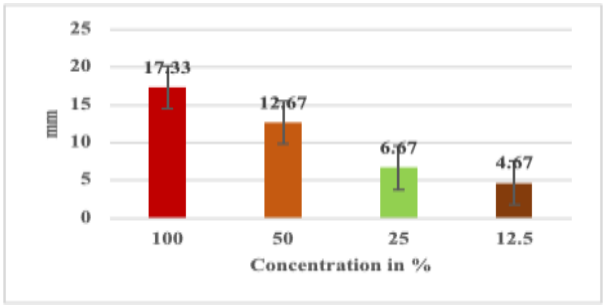

Figure 4. Antimicrobial activity of CCEO against *Candida tropicalis*

Figure 4 depicts the CCEO's antimicrobial action against *C. tropicalis*. The concentration that exhibited the best antimicrobial action was 100% (17.33±0.58 mm). Gram-negative microorganisms are less susceptible to the CCEO, which may be because the cell wall is surrounded by an outer membrane that prevents hydrophobic substances from diffusing through its lipopolysaccharide coating [20, 21].

Antimicrobial activity against Gram-positive bacteria

Figure 5 depicts the CCEO antimicrobial activity against *S. aureus*. The concentration that exhibited the best antimicrobial action was 100 % (7.67±0.58 mm).

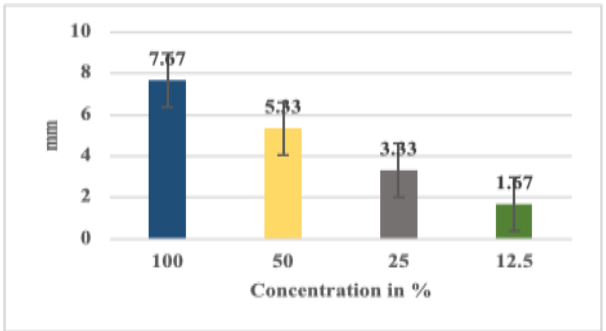

Figure 5. Antimicrobial activity of CCEO against *Staphylococcus aureus*

Figure 5 depicts the CCEO antimicrobial action against *S. aureus*. The concentration that exhibited the best antimicrobial action was 100% (7.67±0.58 mm).

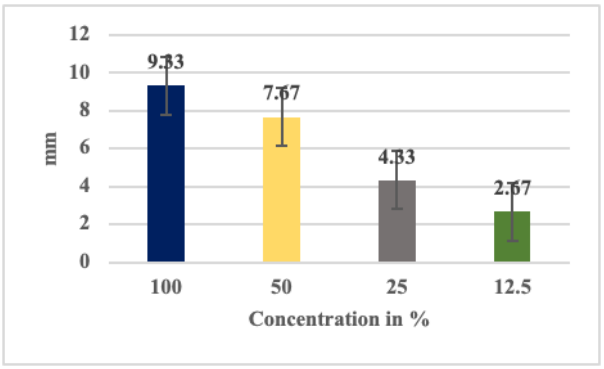

Figure 6. Antimicrobial activity of CCEO against *Micrococcus luteus*

The antimicrobial activity of CCEO against *M. luteus* showed Figure 6. The best antimicrobial activity was found with 100 % concentration (9.33±0.58 mm).

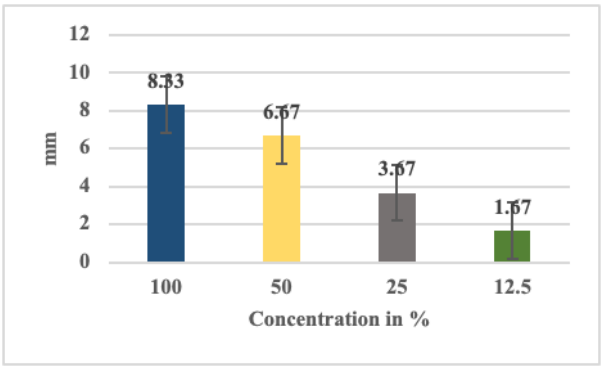

Figure 7. Antimicrobial activity of CCEO against *Listeria monocytogenes*

The antimicrobial activity of CCEO against *M. luteus* showed Figure 7. The best antimicrobial activity was found with 100 % concentration (8.33±0.58 mm).

Antimicrobial activity against Gram-negative bacteria

Another well-known eucalypt with antibacterial, antifungal, analgesic, and anti-inflammatory qualities is *C. citriodora* [22, 23]. The antimicrobial activity of CCEO against *E. coli* showed Figure 8. The best antimicrobial activity was found with 100 % concentration (12.67±0.58 mm).

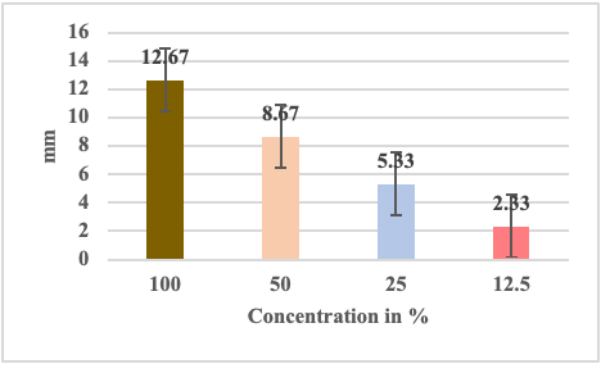

Figure 8. Antimicrobial activity of CCEO against *Escherichia coli*

The antimicrobial activity of CCEO against *P. putida* showed Figure 9. The best antimicrobial activity was found with 100 % concentration (10.33±0.58 mm). Natural product with a variety of biological characteristics is *C. citriodora* essential oil. It is one of the species that is most frequently used in the modern food, cosmetic, and pharmaceutical sectors [24]. The findings from the disc diffusion technique used to assess the antifungal potential of *C. citriodora* essential oil are compiled in the study of Tolba [24]. According to the findings, all of the dermatophytes tested in the current research had their growth successfully inhibited by *E. citriodora* essential oil. With rising oil concentrations (10, 20, 30 µL/disc), the zone of inhibition grew larger.

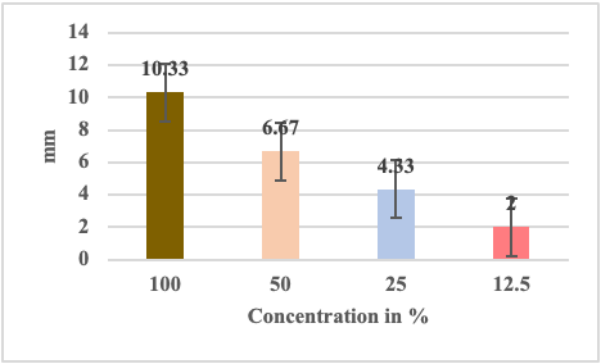

Figure 9. Antimicrobial activity of CCEO against *Pseudomonas putida*

The antimicrobial activity of CCEO against *E. aerogenes* showed Figure 10. The best antimicrobial activity was found with 100 %

concentration (9.67±0.58 mm). According to our findings, fungi, and bacteria were the two types of microorganisms that the essential oil of eucalyptus was most potent against [18, 25–37].

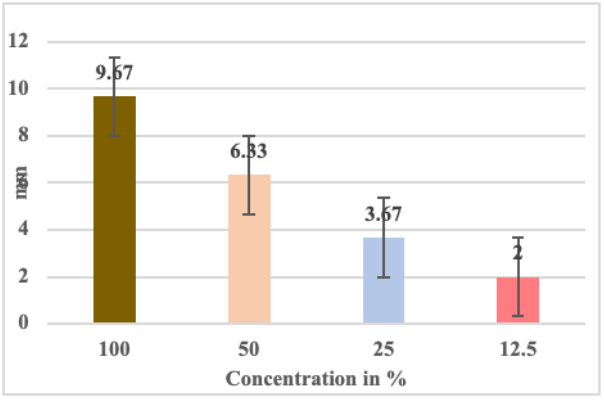

Figure 7. Antimicrobial activity of CCEO against *Enterobacter aerogenes*

4. Conclusions

This research has demonstrated that the essential oil of the Brazilian-grown *Corymbia citriodora* has a sizable amount of activity against a variety of microorganisms, including human pathogens, bacteria that cause food poisoning and spoilage, and the Candida blastomycete opportunistic fungi. These findings support *C. citriodora* essential oil's potential for use in the food and pharmaceutical sectors, as well as its potential value as a natural antimicrobial agent for the treatment of a variety of infectious diseases.

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References

1. Gilles, M., Zhao, J., An, M., Agboola, S., Chemical composition and antimicrobial properties of essential oils of three Australian *Eucalyptus* species. Food Chem. 2010, https://doi.org/10.1016/j.foodchem.2009.07.021
2. Silva, S.M., Abe, S.Y., Murakami, F.S., Frensch, G., Marques, F.A., Nakashima, T., Essential Oils from Different Plant Parts of *Eucalyptus cinerea* F. Muell. ex Benth. (Myrtaceae) as a Source of 1,8-Cineole and Their Bioactivities. Pharmaceuticals. 2011, https://doi.org/10.3390/ph4121535
3. Dhakad, A.K., Pandey, V.V., Beg, S., Rawat, J.M., Singh, A., Biological, medicinal and toxicological significance of *Eucalyptus* leaf essential oil: a review: Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil. J. Sci. Food Agric. 2018, https://doi.org/10.1002/jsfa.8600
4. Miguel, M., Gago, C., Antunes, M., Lagoas, S., Faleiro, M., Megías, C., Cortés-Giraldo, I., Vioque, J., Figueiredo, A., Antibacterial, Antioxidant, and Antiproliferative Activities of Corymbia citriodora and the Essential Oils of Eight Eucalyptus Species. Medicines. 2018, https://doi.org/10.3390/medicines5030061
5. Hutchings, A., Scott, A.H., Zulu medicinal plants: an inventory. University of Natal Press, Pietenmaritzburg, South Africa, 1996
6. Malaspina, P., Papaianni, M., Ranesi, M., Polito, F., Danna, C., Aicardi, P., Cornara, L., Woo, S.L., De Feo, V., *Eucalyptus cinerea* and *E. nicholii* by-Products as Source of Bioactive Compounds for Agricultural Applications. Plants. 2022, https://doi.org/10.3390/plants11202777
7. Luqman, S., Dwivedi, G.R., Darokar, M.P., Kalra, A., Khanuja, S.P.S., Antimicrobial activity of *Eucalyptus citriodora* essential oil, Int. J. Essent. Oil Ther. 2008, 2, 69
8. Fourmy, D., Recht, M.I., Blanchard, S.C., Puglisi, J.D., Structure of the A Site of *Escherichia coli* 16 S Ribosomal RNA Complexed with an Aminoglycoside Antibiotic. Science. 1996, https://doi.org/10.1126/science.274.5291.1367
9. McDevitt, D., Rosenberg, M., Exploiting genomics to discover new antibiotics, Trends Microbiol., 2001, https://doi.org/10.1016/S0966-842X(01)02235-1
10. Walsh, C.T., Fisher, S.L., Park, I.-S., Prahalad, M., Wu, Z., Bacterial resistance to vancomycin: five genes and one missing hydrogen bond tell the story. Chem. Biol. 1996, https://doi.org/10.1016/S1074-5521(96)90079-4
11. Maxwell, A., DNA gyrase as a drug target. Biochem. Soc. Trans. 1999, https://doi.org/10.1042/bst027a003b
12. Le Thomas, I., *In vivo* selection of a target/efflux double mutant of *Pseudomonas aeruginosa* by ciprofloxacin therapy. J. Antimicrob. Chemother. 2001, https://doi.org/10.1093/jac/48.4.553
13. Beauclerk, A.A.D., Cundliffe, E., Sites of action of two ribosomal RNA methylases responsible for resistance to aminoglycosides. J. Mol. Biol. 1987, https://doi.org/10.1016/0022-2836(87)90349-4
14. White, T.C., Marr, K.A., Bowden, R.A., Clinical, Cellular, and Molecular Factors That Contribute to Antifungal Drug Resistance. Clin. Microbiol. Rev. 1998, https://doi.org/10.1128/CMR.11.2.382
15. Cowen, L.E., Anderson, J.B., Kohn, L.M., Evolution of Drug Resistance in *Candida Albicans*. Annu. Rev. Microbiol. 2002,

https://doi.org/10.1146/annurev.micro.56.012302.160907
16. Tenover, F.C., Mechanisms of Antimicrobial Resistance in Bacteria. Am. J. Med. 2006, https://doi.org/10.1016/j.amjmed.2006.03.011
17. Blanchard, J.S., Molecular mechanisms of drug resistance in *Mycobacterium tuberculosis*. Annu. Rev. Biochem. 1996, https://doi.org/10.1146/annurev.bi.65.070196.001243
18. Ramezani, H., Singh, H.P., Batish, D.R., Kohli, R.K., Dargan, J.S., Fungicidal effect of volatile oils from *Eucalyptus citriodora* and its major constituent citronellal. N. Z. Plant Prot. 2002, https://doi.org/10.30843/nzpp.2002.55.3900
19. Lee, D.-S., Kang, M.-S., Hwang, H.-J., Eom, S.-H., Yang, J.-Y., Lee, M.-S., Lee, W.-J., Jeon, Y.-J., Choi, J.-S., Kim, Y.-M., Synergistic effect between dieckol from *Ecklonia stolonifera* and β-lactams against methicillin-resistant Staphylococcus aureus. Biotechnol. Bioprocess Eng. 2008, https://doi.org/10.1007/s12257-008-0162-9
20. Vaara, M., Agents that increase the permeability of the outer membrane. Microbiol. Rev. 1992, https://doi.org/10.1128/mr.56.3.395-411.1992
21. Sikkema, J., de Bont, J.A., Poolman, B., Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 1995, https://doi.org/10.1128/mr.59.2.201-222.1995
22. Lis-Balchin, M., Deans, S.G., Eaglesham, E., Relationship between bioactivity and chemical composition of commercial essential oils. Flavour Fragr. J. 1998, https://doi.org/10.1002/(SICI)1099-1026(199803/04)13:2<98::AID-FFJ705>3.0.CO;2-B
23. Silva, J., Abebe, W., Sousa, S.M., Duarte, V.G., Machado, M.I.L., Matos, F.J.A., Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. J. Ethnopharmacol. 2003, https://doi.org/10.1016/j.jep.2003.09.007
24. Tolba, H., Moghrani, H., Benelmouffok, A., Kellou, D., Maachi, R., Essential oil of Algerian *Eucalyptus citriodora*: Chemical composition, antifungal activity. J. Mycol. Médicale. 2015, https://doi.org/10.1016/j.mycmed.2015.10.009
25. Ramsewak, R.S., Nair, M.G., Stommel, M., Selanders, L., *In vitro* antagonistic activity of monoterpenes and their mixtures against 'toe nail fungus' pathogens. Phytother. Res. 2003, https://doi.org/10.1002/ptr.1164
26. O. Oyedeji, A., Ekundayo, O., Olawore, O.N., Adeniyi, B.A., Koenig, W.A., Antimicrobial activity of the essential oils of five *Eucalyptus* species growing in Nigeria. Fitoterapia. 1999, https://doi.org/10.1016/S0367-326X(99)00083-0
27. Ahmad, I., Beg, A.Z., Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol. 2001, https://doi.org/10.1016/S0378-8741(00)00335-4
28. Sartorelli, P., Marquioreto, A.D., Amaral-Baroli, A., Lima, M.E.L., Moreno, P.R.H., Chemical composition and antimicrobial activity of the essential oils from two species of Eucalyptus. Phytother. Res. 2007, https://doi.org/10.1002/ptr.2051
29. Schelz, Z., Molnar, J., Hohmann, J., Antimicrobial and antiplasmid activities of essential oils. Fitoterapia. 2006, https://doi.org/10.1016/j.fitote.2006.03.013
30. Takahashi, T., Kokubo, R., Sakaino, M., Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculata*. Lett. Appl. Microbiol. 2004, https://doi.org/10.1111/j.1472-765X.2004.01538.x
31. Delaquis, P., Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food Microbiol. 2002, https://doi.org/10.1016/S0168-1605(01)00734-6
32. Shahi, S.K., Shukla, A.C., Bajaj, A.K., Banerjee, U., Rimek, D., Midgely, G., Dikshit, A., Broad Spectrum Herbal Therapy against Superficial Fungal Infections. Skin Pharmacol. Physiol. 2000, https://doi.org/10.1159/000029909
33. Rai, Qureshi, S., Pandey, A.K., In vitro susceptibility of opportunistic Fusarium spp. to essential oils. Mycoses. 1999, https://doi.org/10.1046/j.1439-0507.1999.00267.x
34. Pattnaik, S., Subramanyam, V.R., Kole, C., Antibacterial and antifungal activity of ten essential oils in vitro. Microbios., 1996, 86, 237–246
35. Egawa, H., Tsutsui, O., Tatsuyama, K., Hatta, T., Antifungal substances found in leaves of Eucalyptus species. Experientia. 1977, https://doi.org/10.1007/BF01951263
36. Salari, M.H., Amine, G., Shirazi, M.H., Hafezi, R., Mohammadypour, M., Antibacterial effects of Eucalyptus globulus leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. Clin. Microbiol. Infect. 2006, https://doi.org/10.1111/j.1469-0691.2005.01284.x
37. Wilkinson, J.M., Cavanagh, H.M.A., Antibacterial activity of essential oils from Australian native plants. Phytother. Res. 2005, https://doi.org/10.1002/ptr.1716