Journal of Pre-Clinical and Clinical Research 2021, Vol 15, No 3, 116-120
www.jpccr.eu

RESEARCH ARTICLE

Additivity interactions between fluconazole and citrus essential oils to *Aspergillus fumigatus*

Paula Wróblewska-Łuczka[1,A-D,F], **Jarogniew Łuszczki**[1,A,C,E-F]

[1] *Department of Pathophysiology, Medical University, Lublin, Poland*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Wróblewska-Łuczka P, Łuszczki J. Additivity interactions between fluconazole and citrus essential oils to *Aspergillus fumigatus*. J Pre-Clin Clin Res. 2021; 15(3): 116–120. doi: 10.26444/jpccr/140077

Abstract

Introduction. *Aspergillus fumigatus* is the most common pathogen causing allergic bronchopulmonary mycosis. The pathogenic capacity of *Aspergillus fumigatus* is related to its thermal tolerance and the small size of the spores which enables transfer to the respiratory tract. In the case of fungal diseases, their treatment is based on fungicidal antibiotics, such as fluconazole. Due to the growing problem of drug resistance, new therapeutic solutions are sought, especially of natural origin. Essential oils, due to their anti-bacterial, anti-fungal, anti-inflammatory and immunostimulatory properties, constitute interesting research material in the fight against mould.

Objective. The aim of the study was to assess the type of pharmacodynamic interactions between fluconazole and selected essential oils: lemon, orange, tangerine, and grapefruit in an *in vitro* study against *Aspergillus fumigatus*. Isobolographic analysis of the results allowed determining the type of interactions between fluconazole and the tested essential oils.

Results. According to the research results, a IC_{50} dose of fluconazole versus *Aspergillus fumigatus* IC_{50} = 1.87±0.88 mg/ml. The most active essential oil was lemon oil, which at the concentration of 4% in medium completely inhibited the growth of *Aspergillus fumigatus*. Tangerine essential oil is the least active against *A. fumigatus*. Isobolographic analysis of the interactions between fluconazole and essential oils showed additive interactions for the combination of fluconazole with lemon, orange and grapefruit ols, and an additive interaction with a tendency to synergism for the combination of fluconazole with tangerine oil.

Conclusions. Isobographic analysis can contribute to the introduction of natural substances into the therapy of many diseases.

Key words

Aspergillus, essential oils, moulds, isobolographic analysis, fluconazole

Abbreviations

EO – essential oil; **IC_{50}** – median inhibitory concentration; **$IC_{50\,mix}$** – median inhibitory concentration for the mixture of the tested substances; **PDA** – Potato Dextrose Agar medium

INTRODUCTION AND OBJECTIVE

Aspergillus fumigatus is the most common pathogen causing allergic bronchopulmonary mycosis. Moreover, it is often isolated from the respiratory tract in patients with asthma who do not meet the criteria for allergic bronchopulmonary aspergillosis [1, 2], and from patients with respiratory diseases other than asthma, including cystic fibrosis [3, 4], chronic obstructive pulmonary disease [5], tuberculosis associated with fibrosis [6], and sometimes in the respiratory tract of healthy people [7]. The pathogenic capacity of *Aspergillus fumigatus* is related to its thermal tolerance, which allows it to grow at the temperature of the human body, and the small size of the spores (about 2–3 μm), which enables transfer to the respiratory tract [8]. *Aspergillus fumigatus* causes aspergillosis in immunocompromised patients. Invasive aspergillosis is still associated with a high mortality rate, ranging from 30% – 90% of patients [9, 10]. It is estimated that *Aspergillus fumigatus* is the etiological factor of various forms of aspergillosis in over 200,000 patients annually [11].

Address for correspondence: Paula Wróblewska-Łuczka, Department of Pathophysiology, Medical University, Jaczewskiego 8b, 20-090 Lublin, Poland
E-mail: paula.luczka@umlub.pl

Received: 14.06.2021; accepted: 08.07.2021; first published: 19.07.2021

In the case of fungal diseases, their treatment is based on fungicidal antibiotics, such as fluconazole [12, 13]. Due to the growing problem of drug resistance, new therapeutic solutions are sought for, especially those of natural origin. Essential oils, due to their anti-bacterial, anti-fungal, anti-inflammatory and immunostimulatory properties, constitute interesting research material in the fight against mould [14–18].

For a very long time, compounds of natural origin were the only medicinal substances available to humans. They still play an important role, especially in self-healing processes. The use of herbs and plants as medicines and nutraceuticals is now an important area of research. About 80% of the world's population believe in the health benefits of plants [19, 20]. Active substances of natural origin are a rich source of compounds with different chemical structures, often showing different biological activities. They are substances showing a high affinity for binding with, e.g. enzymes or receptors found in living organisms. With time, drugs of natural origin have been replaced by their semi-synthetic and synthetic derivatives. Currently, new drugs with interesting chemical structures and previously unknown mechanisms of action are sought. The introduction of new drugs for use is a long-term process due to the duration of preclinical and clinical trials. The search for new drugs results from the depletion of current treatment

options, including bacterial and fungal infections, especially in the case of multi-drug resistant strains [21, 22]. Antibiotic resistance is both a medical and an economic problem, caused by the abuse of many antimicrobial drugs. Essential oils may be an effective alternative to antibiotics when fighting drug-resistant microorganisms [17, 18, 23]. Many essential oils and plant extracts have strong anti-microbial properties. Due to the increasing popularity of natural medicines, as well as the market demand for such products, it seems very important to study the properties of plants and their secondary metabolites, including essential oils [24, 25].

Isobographic analysis is mainly used to evaluate drug interactions in animal studies. In *in vitro* microbiological studies it has been used only several times, mainly to assess the interaction of fluconazole with citrus essential oils against *Cladosporium cladosporioides* [26], the interaction of antibiotics in the treatment of *Aspergillus fumigatus* [27–30], or the assessment of interactions between antibiotics in the treatment of mycobacterial infections *Mycobacterium* [31].

The aim of the study was to assess the nature of the pharmacodynamic interactions between fluconazole and the essential oils: lemon, orange, tangerine and grapefruit in *in vitro* studies against *Aspergillus fumigatus*.

MATERIALS AND METHOD

Plate tests. The research was carried out using the method of plate cultivation on PDA – Potato Dextrose Agar (Biocorp) medium with 1% Tween 20. Cultures were carried out for 8 days at 37 °C. Fluconazole was tested against *Aspergillus fumigatus* at concentrations ranging from 0.05–3.3 mg/ml and the activity of essential oils added to PDA medium at concentrations ranging from 1–30%. The following essential oils were used in the research: lemon (*Citrus limon*), orange (*Citrus aurantium*), tangerine (*Citrus reticulata*) and grapefruit *(Citrus paradisi*). Commercially available essential oils (Avicenna-Oil, Poland) were used for the research. The test substance was added to the medium, PDA with 1% Tween 20, in an appropriate amount. Each of the assays was performed in triplicate. After 8 days of growth, the size of the colony was measured in comparison with the control, which allowed to determine the percentage of inhibition of fungal growth caused by the addition of fluconazole or essential oils to the medium [32,33].

Isobolographic analysis. The obtained results were used to determine the 'dose-effect' curve of the dependence of the concentration of the tested substances or their mixtures in the medium to the percentage of fungal growth inhibition according to the log-probit method, according to Litchfield and Wilcoxon [34]. The IC_{50} (median inhibitory concentration, 50% inhibitory effect on the growth of *A.fumigatus*) doses of the test substances were determined. The test for parallelism of concentration-response effect lines for fluconazole and each of the tested essential oils was performed. This had an impact on the appearance of the additivity line on the isobologram. The effect of a mixture of fluconazole and essential oils in a constant ratio of 1:1 on *Aspergillus fumigatus* was also assessed by the plate culture method. The isobolographic analysis of the obtained results was performed [35–37], which allowed determination of the interaction between fluconazole and the tested essential oils.

RESULTS

Based on the plate tests for *Aspergillus fumigatus*, it was shown that fluconazole in the medium at a concentration of up to 0.4 mg/ml did not cause any inhibition of fungal growth, while the concentration of 3.3 mg/ml caused a growth inhibition of 68% (Fig. 1).

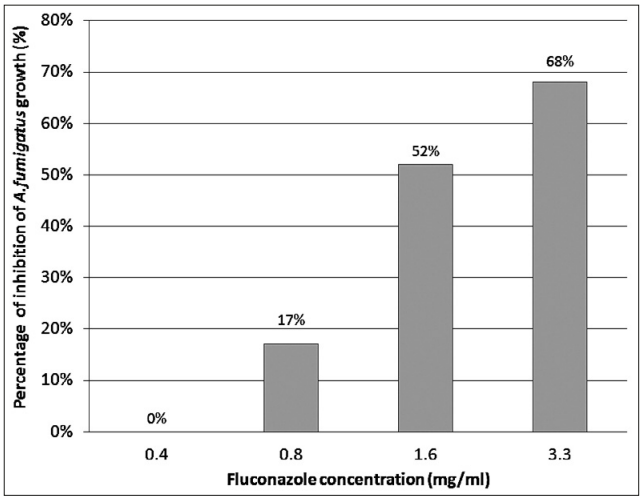

Figure 1. Fluconazole concentration (mg/ml) versus percentage inhibition of *Aspergillus fumigatus* growth

In the case of essential oils, it was determined that none of the tested oils inhibited the growth of *A. fumigatus* at a concentration of 1%. Lemon essential oil was the most active at a concentration of 4% and higher in the medium, it completely inhibited the growth of *Aspergillus fumigatus*. Orange and grapefruit essential oils showed weaker activity than lemon oil, at a concentration of 10% they completely inhibited the growth of *A. fumigatus*. Tangerine essential oil was the weakest in relation to *A. fumigatus*, the concentration of 15% of the oil in the medium caused a complete inhibition of the growth of this fungus (Fig. 2).

Based on the obtained results, using the log-probit method according to Litchfield and Wilcoxon (1949) [34], graphs were drawn for the 'dose-effect' relationship of the tested essential oils, fluconazole and their 1:1 mixture against *Aspergillus fumigatus* (Fig. 3). IC_{50} doses were determined for fluconazole and the tested essential oils against *A. fumigatus* (Tab. 1).

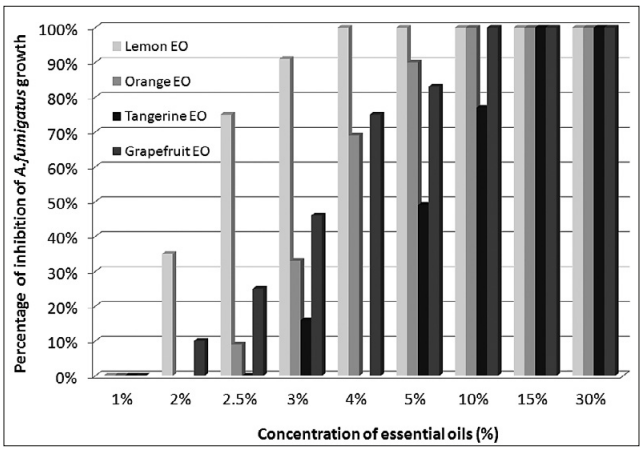

Figure 2. Dependence of the concentration of the studied essential oils in the medium on the percentage of growth inhibition of *Aspergillus fumigatus*

Table 1. Antifungal effect of fluconazole and 4 essential oils: lemon, orange, tangerinen and grapefruit administrated singly in the *Aspergillus fumigatus*

Substance	IC50 ± S.E.
Fluconazole	IC50 = 1,87±0,88 mg/ml
Lemon EO	IC50 = 2,17±0,29 %
Orange EO	IC50 = 3,50±0,45 %
Tangerine EO	IC50 = 5,94±1,77 %
Grapefruit EO	IC50 = 3,24±0,60 %

On the basis of plate tests for *Aspergillus fumigatus*, IC_{50} values were determined for a mixture of the tested essential oil and fluconazole in a constant dose ratio of 1:1 and for lemon oil: $IC_{50\,mix}$=1.54 ± 0.24 mg%/ml, for orange oil: $IC_{50\,mix}$=1.65 ± 0.28 mg%/ml, for tangerine oil: $IC_{50\,mix}$=2.36 ± 0.47 mg%/ml and grapefruit: $IC_{50\,mix}$=1.89 ± 0.35 mg%/ml. Isobolograms were created showing the interactions of the studied essential oils with fluconazole in a constant 1:1 ratio. Isobolographic analysis showed that the combination of fluconazole with orange, lemon and grapefruit essential oil had an additive

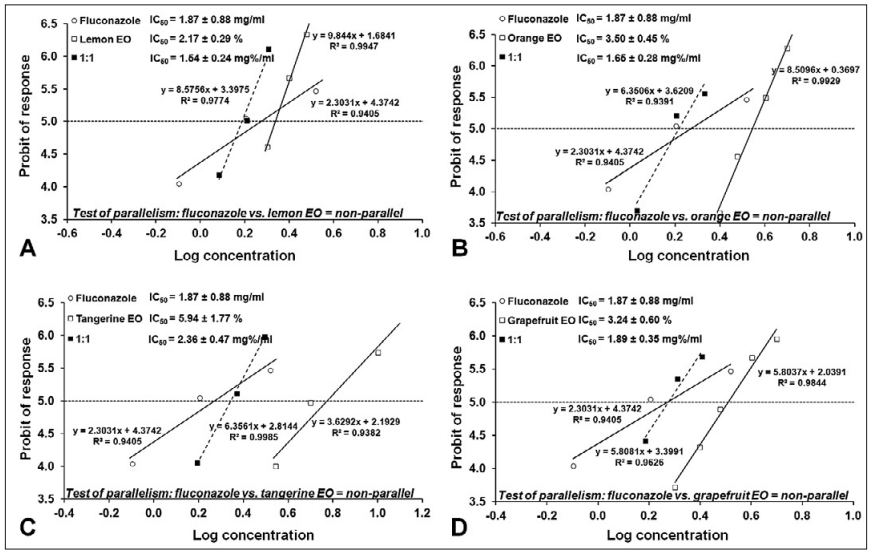

Figure 3. Log-probit concentration-response of the concentrations of fluconazole, essential oil and their combinations on the inhibition of *Aspergillus fumigatus* colony growth. Log-probit concentration-response relationship lines for fluconazole and 4 tested essential oils administered alone and in combination at the fixed-ratio of 1:1, illustrating the antifungal effects of the drugs in *Aspergillus fumigatus* measured *in vitro* by plate method

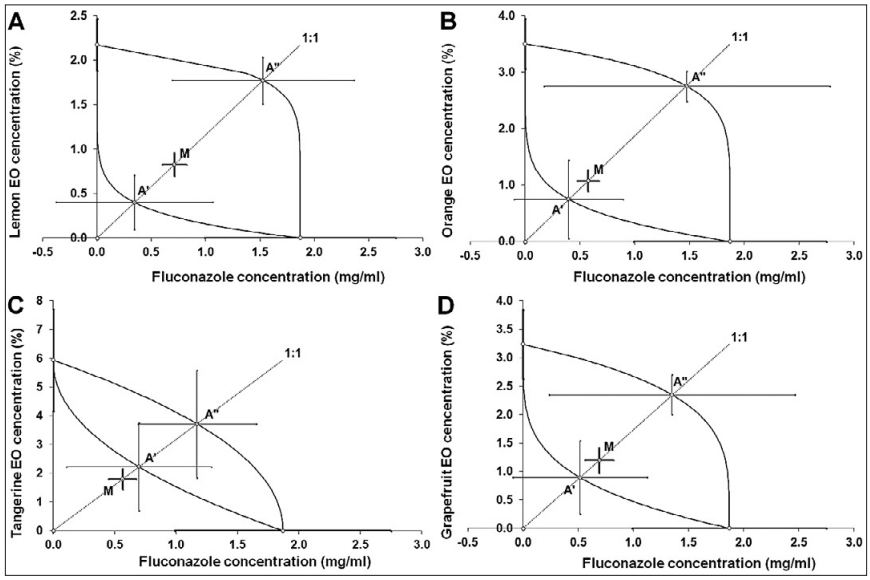

Figure 4. Isobolograms showing interactions between fluconazole and essential oils in relation to *Aspergillus fumigatus*. Isobolograms showing additive interactions between fluconazole and tested essential oils for *Aspergillus fumigatus*. The median inhibitory concentrations (IC_{50}) for fluconazole and the tested essential oils (lemon (A), orange (B), tangerine (C) and grapefruit (D)) are plotted on the X- and Y-axes, respectively. The solid lines on the X and Y axes represent the S.E. for the IC_{50} values for the studied drugs, when administered alone. The lower and upper isoboles of additivity represent the curves connecting the IC_{50} values for fluconazole and the tested essential oils administered alone, if their concentration-response relationships were non-parallel. The dotted line starting from the point (0, 0) corresponds to the fixed-ratio of 1:1 for the combination of fluconazole with each of the tested essential oils. The points A′ and A″ depict the theoretically calculated $IC_{50\,add}$ values for both, lower and upper isoboles of additivity. The point M represents the experimentally-derived $IC_{50\,exp}$ value for total dose of the mixture expressed as proportions of fluconazole and each of the tested essential oils that produced a 50% antifungal effect in *Aspergillus fumigatus*, as measured *in vitro* by plate tests

interaction, and with tangerine essential oil – an additive interaction with a tendency to synergism in plate tests for *Aspergillus fumigatus* (Fig. 4).

DISCUSSION

Fluconazole is used in some experimental studies as a standard anti-fungal drug against, among others: *Aspergillus niger, Aspergillus flavus, Alternaria alternata* [38–40]. The minimum concentration of fluconazole that inhibited the growth of *Aspergillus fumigatus* in 50% was 16 μg/ml [41]. However, the minimum concentration inhibiting the growth of 80% of *Aspergillus fumigatus* for fluconazole was from 64 μg/ml [42]. The results presented in this study showed that the IC_{50} dose of fluconazole, which can be compared with the minimum concentration inhibiting the growth of microorganisms in 50%, is slightly higher than in the presented studies, which may be due to methodological differences, e.g. the use of microtiter plates [41, 42], or shortening breeding time of 5–7 days [42]. According to the results of the current research, the IC_{50} dose of fluconazole versus *Aspergillus fumigatus* IC_{50}=1.87±0,88 mg/ml.

The action of grapefruit oil was species-specific. Lower MIC concentrations were observed for yeasts compared to mould fungi. *Candida* species are more sensitive to the action of grapefruit essential oil compared to *Aspergillus* moulds. Interestingly, grapefruit essential oil showed a stronger antifungal activity compared to pure limonene, which indicated a synergistic effect of all components of the oil. The minimum inhibitory concentration of grapefruit essential oil was determined to be 100 mg/l after a 2-day incubation of *Aspergillus fumigatus* [43]. Tangerine essential oil exhibited a wide anti-fungal spectrum inhibiting the growth of *Aspergillus fumigatus, Aspergillus terreus, Alternaria alternata, Fusarium oxysporum, Penicillum italicum* and *Trichoderma viride* at 750 ppm of oil in PDA medium [44]. After 14 days of incubation, *Aspergillus flavus* growth was inhibited to 18.70% in response to lemon essential oil, and 5.92% to orange essential oil. The essential oils showed a potential inhibitory activity against toxic fungi and inhibited the production of mycotoxins, especially aflatoxin B1 [45]. In other studies, bergamot oil (from the citrus-bergamot orange) was found to be the most effective, inhibiting the growth of *Aspergillus flavus* by 76.51% at a concentration of 5% of the oil in medium. Other active oils were: bitter orange and sweet orange oil (inhibiting mycelium growth in approx. 60% at 5% oil concentration), and then lemon oil (inhibiting mycelium growth in approx. 55% at 5% oil concentration). Tangerine essential oil showed the lowest percentage of mycelial inhibition compared to other citrus essential oils. The observed differences in anti-fungal activity among citrus essential oils may be due to differences in chemical composition, although limonene can be considered the main inhibitory component present in citrus essential oils [32].

The presented research also confirms that tangerine essential oil is the least active against *Aspergillus fumigatus*. The most active was lemon oil, which at the concentration of 4% completely inhibited the growth of *Aspergillus fumigatus*. The differences may result from species differences between the fungi used in the research and those indicated by other authors, and from differences in the composition of the tested essential oils.

The isobolographic analysis of interactions between fluconazole and essential oils against *Aspergillus fumigatus* showed additive interactions for the combination of fluconazole with lemon, orange and grapefruit oils and an additive interaction with a tendency to synergism for the combination of fluconazole with tangerine oil. Other studies for *Cladosporium cladosporioides* showed additive interactions for the combination of fluconazole with orange and grapefruit essential oil and additive with a tendency for synergism for combination of fluconazole with lemon and tangerine essential oils [26]. Additive interactions between fluconazole and essential oils may be related to the fact that both fluconazole and essential oils inhibit the activity of cytochrome P450 (CYP) enzymes in fungi. Cytochrome enzymes are responsible for the synthesis of ergosterol (the main sterol of the fungal cell membrane). Disturbances in ergosterol synthesis cause changes in the fluidity of the cell membrane, thus inhibiting fungal replication [46, 47]. Animal studies have confirmed that limonene, the main component of citrus essential oils, reduces the activity of CYP2B1 and CYP2B6 [48,49]. While fluconazole inhibits: CYP2C9, CYP2C19, CYP3A4 of cytochrome P450 enzymes [47]. Due to the inhibition of other cytochrome enzymes, the complementary effects of fluconazole and the essential oils appear to elicit additive interactions.

CONCLUSIONS

The promising results of the conducted *in vitro* tests encourage and justify the need for further research and analysis. This may contribute to the development of new therapeutic methods of fungal infections using known antifungal drugs, the action of which would be supplemented with active substances of natural origin. Searching for natural substances with anti-fungal activity and determining their interactions with known antifungal drugs may turn out to be an interesting trend in research due to the possibility of limiting the use of antibiotics and their side-effects.

The use of the isobolographic analysis method to assess the interaction of a standard drug in combination with an active substance of natural origin in *in vitro* studies on microorganisms is a fairly new procedure. Isobographic analysis can contribute to introducing natural substances into the therapy of many diseases.

REFERENCES

1. Agbetile J, Fairs A, Desai D, et al. Isolation of filamentous fungi from sputum in asthma is associated with reduced post-bronchodilator FEV1. Clin Exp Allergy. 2012; 42: 782–791. doi: 10.1111/j.1365-2222.2012.03987.x
2. Hérivaux A, Gonçalves SM, Carvalho A, Cunha C. Microbiota-derived metabolites as diagnostic markers for respiratory fungal infections. J Pharm Biomed Anal. 2020; 189: 113473. doi: https://doi.org/10.1016/j.jpba.2020.113473
3. Baxter CG, Moore CB, Jones AM, et al. IgE-mediated immune responses and airway detection of Aspergillus and Candida in adult cystic fibrosis. Chest. 2013; 143: 1351–1357. doi: 10.1378/chest.12-1363
4. Keown K, Reid A, Moore JE, Taggart CC, Downey DG. Coinfection with Pseudomonas aeruginosa and Aspergillus fumigatus in cystic fibrosis. Eur Respir Rev. 2020; 29(158): 200011. doi: 10.1183/16000617.0011-2020
5. Bafadhel M, McKenna S, Agbetile J, et al. Aspergillus fumigatus during stable state and exacerbations of COPD. Eur Respir J. 2014; 43: 64–71. doi: 10.1183/09031936.00162912

6. Dhooria S, Kumar P, Saikia B, et al. Prevalence of Aspergillus sensitisation in pulmonary tuberculosis-related fibrocavitary disease. Int J Tuberc Lung Dis. 2014; 18: 850–855. doi: 10.5588/ijtld.13.0838
7. Wassano NS, Goldman GH, Damasio A. Aspergillus fumigatus. Trends Microbiol. 2020; 28(7): 594–595. doi: 10.1016/j.tim.2020.02.013
8. Kwon-Chung KJ, Sugui JA. Aspergillus fumigatus – what makes the species a ubiquitous human fungal pathogen? PLoS Pathog 2013; 9: e1003743. doi: 10.1371/journal.ppat.1003743
9. van de Veerdonk FL, Gresnigt MS, Romani L, et al. Aspergillus fumigatus morphology and dynamic host interactions. Nat Rev Microbiol. 2017; 15(11): 661–674. doi: 10.1038/nrmicro.2017.90
10. Russo A, Tiseo G, Falcone M, Menichetti F. Pulmonary Aspergillosis: An Evolving Challenge for Diagnosis and Treatment. Infect Dis Ther. 2020; 9(3): 511–524. doi: 10.1007/s40121-020–00315-4
11. Latgé JP, Chamilos G. Aspergillus fumigatus and Aspergillosis in 2019. Clin Microbiol Rev. 2019; 33(1): e00140–18. doi: 10.1128/CMR.00140-18
12. Wiederhold NP. Antifungal resistance: current trends and future strategies to combat. Infect Drug Resist. 2017; 10: 249–259. doi: 10.2147/IDR.S124918
13. Guinea J. Updated EUCAST Clinical Breakpoints against Aspergillus, Implications for the Clinical Microbiology Laboratory. J Fungi (Basel). 2020; 6(4): 343. doi: 10.3390/jof6040343
14. Hocayen PAS, Wendler E, Vecchia DD, et al. The nitrergic neurotransmission contributes to the anxiolytic-like effect of Citrus sinensis essential oil in animal models. Phytother Res. 2019; 33(4): 901–909. doi: 10.1002/ptr.6281
15. Falzon CC, Balabanova A. Phytotherapy: An Introduction to Herbal Medicine. Prim Care. 2017; 44(2): 217–227. doi: 10.1016/j.pop.2017.02.001
16. Colalto C. What phytotherapy needs: Evidence-based guidelines for better clinical practice. Phytother Res. 2018; 32(3): 413–425. doi: 10.1002/ptr.5977
17. Geraci A, Di Stefano V, Di Martino E, et al. Essential oil components of orange peels and antimicrobial activity. Nat Prod Res. 2017; 31(6): 653–659. doi: 10.1080/14786419.2016.1219860
18. Ambrosio CMS, Ikeda NY, Miano AC, et al. Unraveling the selective antibacterial activity and chemical composition of citrus essential oils. Sci Rep. 2019; 9(1): 17719. doi: 10.1038/s41598-019-54084-3
19. Suroowan S, Mahomoodally MF. Herbal Medicine of the 21st Century: A Focus on the Chemistry, Pharmacokinetics and Toxicity of Five Widely Advocated Phytotherapies. Curr Top Med Chem. 2019; 19(29): 2718–2738. doi: 10.2174/1568026619666191112121330
20. Clements ND, Connolly BR, Dicks MA, Mullur RS. The Use of Vitamins, Supplements, Herbs, and Essential Oils in Rehabilitation. Phys Med Rehabil Clin N Am. 2020; 31(4): 685–697. doi: 10.1016/j.pmr.2020.07.010
21. Diniz do Nascimento L, Moraes AAB, Costa KSD, et al. Bioactive Natural Compounds and Antioxidant Activity of Essential Oils from Spice Plants: New Findings and Potential Applications. Biomolecules. 2020; 10(7): 988. doi: 10.3390/biom10070988
22. Yap PS, Yiap BC, Ping HC, Lim SH. Essential oils, a new horizon in combating bacterial antibiotic resistance. Open Microbiol J. 2014; 8: 6–14. doi: 10.2174/1874285801408010006
23. Quirino A, Morelli P, Capua G, et al. Synergistic and antagonistic effects of Citrus bergamia distilled extract and its major components on drug resistant clinical isolates. Nat Prod Res. 2020 Jun; 34(11): 1626–1629. doi: 10.1080/14786419.2018.1522631
24. Wińska K, Mączka W, Łyczko J, et al. Essential Oils as Antimicrobial Agents-Myth or Real Alternative? Molecules. 2019; 24(11): 2130. doi: 10.3390/molecules24112130
25. Yap PS, Lim SH, Hu CP, Yiap BC. Combination of essential oils and antibiotics reduce antibiotic resistance in plasmid-conferred multidrug resistant bacteria. Phytomedicine. 2013; 20(8–9): 710–3. doi: 10.1016/j.phymed.2013.02.013
26. Wróblewska-Łuczka P. Isobolographic in vitro interactions of fluconazole with citrus essential oils against Cladosporium cladosporioides. J Pre Clin Clin Res. 2021. doi: https://doi.org/10.26444/jpccr/132014
27. Elefanti A, Mouton JW, Verweij PE, et al. Amphotericin B- and voriconazole-echinocandin combinations against Aspergillus spp.: Effect of serum on inhibitory and fungicidal interactions. Antimicrob Agents Chemother. 2013; 57(10): 4656–4663. doi: 10.1128/AAC.00597-13
28. Meletiadis J, te Dorsthorst DT, Verweij PE. The concentration-dependent nature of in vitro amphotericin B-itraconazole interaction against Aspergillus fumigatus: isobolographic and response surface analysis of complex pharmacodynamic interactions. Int J Antimicrob Agents. 2006; 28(5): 439–449. doi: 10.1016/j.ijantimicag.2006.07.011
29. Stergiopoulou T, Meletiadis J, Sein T, et al. Isobolographic analysis of pharmacodynamic interactions between antifungal agents and ciprofloxacin against Candida albicans and Aspergillus fumigatus. Antimicrob Agents Chemother. 2008; 52(6): 2196–204. doi: 10.1128/AAC.00735-07
30. Stergiopoulou T, Meletiadis J, Sein T, et al. Comparative pharmacodynamic interaction analysis between ciprofloxacin, moxifloxacin and levofloxacin and antifungal agents against Candida albicans and Aspergillus fumigatus. J Antimicrob Chemother. 2009; 63(2): 343–348. doi: 10.1093/jac/dkn473
31. Ferro BE, Meletiadis J, Wattenberg M, et al. Clofazimine prevents the regrowth of Mycobacterium abscessus and Mycobacterium avium type strains exposed to amikacin and clarithromycin. Antimicrob Agents Chemother. 2015; 60(2): 1097–1105. doi: https://doi.org/10.1128/AAC.02615-15
32. Aloui H, Khwaldia K, Licciardello F, et al. Efficacy of the combined application of chitosan and Locust Bean Gum with different citrus essential oils to control postharvest spoilage caused by Aspergillus flavus in dates. Int J Food Microbiol. 2014; 170: 21–28. doi: https://doi.org/10.1016/j.ijfoodmicro.2013.10.017
33. Budzyńska A, Więckowska-Szakiel M, Kalemba D, et al. The optimization of methods utilized for testing the antibacterial activity of essential oils. Med Dośw Mikrobiol. 2009; 61: 281–287.
34. Litchfield JT Jr, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther. 1949; 96: 99–113.
35. Łuszczki JJ. Isobolographic analysis of interaction between drugs with nonparallel dose-response relationship curves: a practical application. Naunyn Schmiedebergs Arch Pharmacol. 2007; 375: 105–114. https://doi.org/10.1007/s00210-007-0144-z
36. Luszczki JJ, Mazurkiewicz LP, Wroblewska-Luczka P, et al. Combination of phenobarbital with phenytoin and pregabalin produces synergy in the mouse tonic-clonic seizure model: An isobolographic analysis. Epilepsy Res. 2018; 145: 116–122. doi: 10.1016/j.eplepsyres.2018.06.003
37. Luszczki JJ, Zagaja M, Miziak B, et al. Beneficial Combination of Lacosamide with Retigabine in Experimental Animals: An Isobolographic Analysis. Pharmacology. 2018; 101(1–2): 22–28. doi: 10.1159/000480019
38. Riaz T, Abbasi MA, Rehman A, et al. Enzyme inhibitory, antifungal, antibacterial and hemolytic potential of various fractions of Colebrookia oppositifolia. Pak J Pharm Sci. 2017; 30(1): 105–112
39. Linder KA, Kauffman CA, Patel TS, et al. Evaluation of targeted versus universal prophylaxis for the prevention of invasive fungal infections following lung transplantation. Transpl Infect Dis. 2020: e13448. doi: 10.1111/tid.13448
40. Kim JH, Cheng LW, Chan KL, et al. Antifungal Drug Repurposing. Antibiotics (Basel). 2020; 9(11): 812. doi: 10.3390/antibiotics9110812
41. Sun N, Li D, Zhang Y, et al. Repurposing an inhibitor of ribosomal biogenesis with broad anti-fungal activity. Sci Rep. 2017; 7(1): 17014. doi: 10.1038/s41598-017-17147-x
42. Wu J, Ni T, Chai X, et al. Molecular docking, design, synthesis and antifungal activity study of novel triazole derivatives. Eur J Med Chem. 2018; 143: 1840–1846. doi: 10.1016/j.ejmech.2017.10.081
43. Pekmezovic M, Aleksic I, Barac A, et al. Prevention of polymicrobial biofilms composed of Pseudomonas aeruginosa and pathogenic fungi by essential oils from selected Citrus species. Pathog Dis. 2016; 74(8): ftw102. doi: 10.1093/femspd/ftw102
44. Denkova-Kostova R, Teneva D, Tomova T, et al. Chemical composition, antioxidant and antimicrobial activity of essential oils from tangerine (Citrus reticulata L.), grapefruit (Citrus paradisi L.), lemon (Citrus lemon L.) and cinnamon (Cinnamomum zeylanicum Blume). Z Naturforsch C J Biosci. 2020; 76(5–6): 175–185. doi: 10.1515/znc-2020-0126
45. Císarová M, Tančinová D, Medo J, Kačániová M. The in vitro effect of selected essential oils on the growth and mycotoxin production of Aspergillus species. J Environ Sci Health B. 2016; 51(10): 668–674. doi: 10.1080/03601234.2016.1191887
46. Zehetner P, Höferl M, Buchbauer G. Essential oil components and cytochrome P450 enzymes: a review. Flavour and Fragrance Journal. 2019; 34(4): 223–240. doi: https://doi.org/10.1002/ffj.3496
47. Alammari AH, Shoieb SM, Maayah ZH, El-Kadi AOS. Fluconazole Represses Cytochrome P450 1B1 and Its Associated Arachidonic Acid Metabolites in the Heart and Protects Against Angiotensin II-Induced Cardiac Hypertrophy. J Pharm Sci. 2020; 109(7): 2321–2335. doi: 10.1016/j.xphs.2020.03.016
48. Masuda M, Watanabe S, Tanaka M, et al. Screening of furanocoumarin derivatives as cytochrome P450 3A4 inhibitors in citrus. J Clin Pharm Ther. 2018; 43(1): 15–20. doi: 10.1111/jcpt.12595
49. OuYang Q, Tao N, Jing G. Transcriptional profiling analysis of Penicillium digitatum, the causal agent of citrus green mold, unravels an inhibited ergosterol biosynthesis pathway in response to citral. BMC Genomics. 2016; 17(1): 599. doi: 10.1186/s12864-016-2943-4