REVISTA
DESAFIOS
ISSN: 2359-3652
V.12, n.4, julho/2025 - DOI:10.20873/2025_jul_19833

STUDY ON THE EFFECT OF CARVACROL IN ASSOCIATION WITH AMPHOTERICIN B AGAINST *Cryptococcus neoformans* STRAIN (LM-22)

ESTUDO SOBRE O EFEITO DO CARVACROL EM ASSOCIAÇÃO COM ANFOTERICINA B CONTRA A CEPA DE Cryptococcus neoformans (LM-22)

ESTUDIO SOBRE EL EFECTO DEL CARVACROL EN ASOCIACIÓN CON ANFOTERICINA B CONTRA LA CEPA DE Cryptococcus neoformans (LM-22)

Rafaela de Oliveira Nóbrega:

Mestrado em Ciências Naturais e Biotecnologia pelo Programa de Pós-Graduação em Ciências Naturais e Biotecnologia. Universidade Federal de Campina Grande (UFCG). E-mail: rafaelabgaa@gmail.com| Orcid.org/ 0000-0003-2303-9218

Anna Paula de Castro Teixeira

Doutora em Desenvolvimento e Inovação Tecnológica em Medicamentos. Universidade Federal da Paraíba (UFPB). E-mail: annapaula.1993@gmail.com| Orcid.org/ 0000-0002-5252-2850

Francisco Patricio de Andrade Júnior

Doutor em Farmacologia. Graduando em Medicina. Universidade Estadual do Piauí (UESPI). E-mail: juniorfarmacia.ufcg@outlook.com | Orcid.org/ 0000-0003-0681-8439

Edeltrudes de Oliveira Lima

Profª. Drª. Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos. Universidade Federal da Paraíba (UFPB). E-mail: edelolima@yahoo.com.br.| Orcid.org/ 0000-0002-9547-0886

Wylly Araújo de Oliveira

Prof. Dr. Programa de Pós-Graduação em Ciências Naturais e Biotecnologia. Universidade Federal de Campina Grande (UFCG). E-mail: wyllyoliveira@gmail.com| Orcid.org/ 0000-0002-7784-0302

Fillipe de Oliveira Pereira

Prof. Dr. Programa de Pós-graduação em Ciências da Nutrição. Universidade Federal da Paraíba (UFPB). E-mail: fillipefop@yahoo.com.br | Orcid.org/ 0000-0002-3081-4174

V.12, n.4, julho/2025. ISSN n° 2359-3652

Igara Oliveira Lima

Profª. Drº. Programa de Pós-Graduação em Ciências Naturais e Biotecnologia. Universidade Federal de Campina Grande (UFCG). E-mail: igara.oliveira@professor.ufcg.edu.br | Orcid.org/ 0000-0002-1418-431X

REVISTA DESAFIOS ISSN: 2359-3652

ABSTRACT:

Cryptococcus neoformans is an encapsulated yeast, etiologic agent of cryptococcosis, an opportunistic and systemic fungal infection, frequent in patients with compromised immune systems. Considering the therapeutic potential of phytochemicals derived from natural products they are fast becoming promising alternatives for both fungal infections and other diseases. The carvacrol is found in various plant essential oils, and possesses many antifungal and antibacterial activities. The study aims to evaluate the in vitro antifungal activity of the carvacrol against *Cryptococcus neoformans*. An antifungal activity investigation was performed using microbial death kinetics, and studying carvacrol in associations with amphotericin B and their effect on *C. neoformans* micromorphology. Using Time to Kill method, our results show that carvacrol inhibits fungal growth, and presents indifferent effects when in association with amphotericin B. Carvacrol also promoted capsule thickness reductions. Under the conditions evaluated carvacrol presented antifungal activity against *C. neoformans* strain LM-22.

KEYWORDS: Carvacrol; amphotericin B; antifungal activity.

RESUMO:

Cryptococcus neoformans é uma levedura encapsulada e agente etiológico da criptococose, uma infecção fúngica oportunista e sistêmica frequente em pacientes com sistemas imunológicos comprometidos. Considerando o potencial terapêutico dos fitoquímicos derivados de produtos naturais, estes estão se tornando alternativas promissoras tanto para infecções fúngicas quanto para outras doenças. O carvacrol, mais especificamente, é encontrado em vários óleos essenciais de plantas e possui atividades antifúngicas e antibacterianas. O estudo tem como objetivo avaliar a atividade antifúngica in vitro do carvacrol contra Cryptococcus neoformans. Uma investigação da atividade antifúngica foi realizada usando cinética de morte microbiana, estudando o carvacrol em associações com anfotericina B e seu efeito na micromorfologia de C. neoformans. Utilizando o método Time to Kill, nossos resultados mostram que o carvacrol inibe o crescimento fúngico e apresenta efeitos indiferentes quando em associação com anfotericina B. O carvacrol também promoveu reduções na espessura da cápsula. Sob as condições avaliadas, o carvacrol apresentou atividade antifúngica contra a cepa LM-22 de C. neoformans.

PALAVRAS-CHAVE: *Carvacrol; anfotericina B; atividade antifúngica.*

RESUMEN:

Cryptococcus neoformans es una levadura encapsulada, agente etiológico de la criptococosis, una infección fúngica oportunista y sistémica frecuente en pacientes con sistemas inmunológicos comprometidos. Considerando el potencial terapéutico de los fitoquímicos derivados de productos naturales, están emergiendo como alternativas prometedoras tanto para infecciones fúngicas como para otras enfermedades. El carvacrol se encuentra en varios aceites esenciales de plantas y posee diversas actividades antifúngicas y antibacterianas. El estudio tiene como objetivo evaluar la actividad antifúngica in vitro del carvacrol contra

Cryptococcus neoformans. Se realizó una investigación de la actividad antifúngica utilizando cinética de muerte microbiana y estudiando el carvacrol en asociación con anfotericina B y su efecto sobre la micromorfología de C. neoformans. Utilizando el método de Tiempo hasta la Muerte, nuestros resultados muestran que el carvacrol inhibe el crecimiento fúngico y presenta efectos indiferentes cuando se asocia con anfotericina B. El carvacrol también promovió reducciones en el grosor de la cápsula. Bajo las condiciones evaluadas, el carvacrol mostró actividad antifúngica contra la cepa LM-22 de C. neoformans.

Palabras clave: *Carvacrol; anfotericina B; actividad antifúngica.*

INTRODUCTION

Between January 2000 and December 2012, 5,755 deaths from cryptococcosis were recorded in Brazil (Soares *et al*., 2019). In AIDS patients in Brazil, *Cryptococcus* spp. is the sixth leading cause of opportunistic infections, overtaking *Candida* spp., *Mycobacterium* spp., *Pneumocystis carinii*, *Toxoplasma gondii*, and the herpes simplex virus (Rathore *et al*., 2022). In Africa, cryptococcal meningitis has passed tuberculosis as the most lethal opportunistic infection occurring in AIDS patients (Mcdonald *et al*., 2012).

Treatment for patients with cryptococcosis involves a combination of drugs: usually amphotericin B with flucytosine or fluconazole. The strategies differ in accordance with the immune deficiency and infection characteristics. For example, initial treatment for meningitis, disseminated, and moderate to severe pulmonary infection is a combination of lipid amphotericin B and 5-flucytosine, followed by a regime involving fluconazole (Spadari *et al*., 2020).

In successful antifungal treatment, combination antifungal therapy is often an essential alternative. In addition to reducing the costs of treatment, a drug combination acting in synergy can make it possible to reduce the dosages of each drug, their toxicity, and the potential for drug resistance (Lira *et al*., 2020). Strains resistant to conventional drugs motivate the search for antifungal agents as alternatives. Plant-derived compounds are important sources of new therapeutic molecules and often appear as alternatives or complements to conventional treatments (Ahn, 2017).

Pharmacological studies increasingly concentrate on this area, elucidating the mechanisms, toxicities, and activities observed in chemical substances of natural origin which display antimicrobial, antiparasitic, insecticide, protozoan, and antioxidant activity (Lira *et al*., 2020).

Carvacrol is a phytoconstituent present in certain essential oils, for example, in Origanum vulgare, where it is a major component (Khan *et al*., 2019) According to

Nóbrega *et al*. (2016), carvacrol presents relevant biological activity and is a promising molecule in the search for antifungal agents, having already presented activity against strains of *C. neoformans*. The present work continues that of Nóbrega *et al*. (2016). Although it is a substance widely studied for its antifungal potential, there is a notable lack of research on the association of carvacrol with antifungal drugs, such as amphotericin B, especially in the context of *Cryptococcus* pathogens, as well as on morphological changes in the fungal capsule. Given this gap, the present study aimed to evaluate the effect of carvacrol, both alone and in combination with amphotericin B, on the capsule of *Cryptococcus neoformans* yeast.

METHODOLOGY

Fungal strains

Cryptococcus neoformans strain (LM-22) was maintained in Sabouraud Dextrose Agar at 28-30°C (room temperature) during experiments, and stored under refrigeration at 4ºC.

Culture medium and synthetic antifungal

Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth and Agar-corn flour (DIFCO - Laboratories Ltd) culture media were used. The preparations were performed according to the manufacturer's instructions. Amphotericin B and carvacrol were commercially acquired through Sigma Aldrich.

Inoculum

Preparation of the inoculum was realized using a recent culture of *C. neoformans* strain LM-22, previously cultivated in sterile tubes containing tilted SDA, and incubated at 35°C for 72 hours to achieve satisfactory growth. The fungal colonies were then suspended in 5 mL of sterile saline solution (0.85 w/v). The resulting suspension was stirred gently and adjusted according to tube turbidity, number 0.5 on the McFarland scale, which corresponds to 5 x 106 CFU/mL (Cleeland and Squires, 1991).

Time to microbial death

Study testing drugs separately

We performed time to kill testing with carvacrol and amphotericin B to verify their individual activities over time against the *C. neoformans* strain. Carvacrol [41.5 µg/mL (MIC/2), 81 µg/mL (MIC), and 162 µg/mL (2xMIC)], and amphotericin B [0.5

µg/mL (MIC/2), 1 µg/mL (MIC), and 2 µg/mL (2xMIC)] were tested separately to assess both their fungistatic and fungicidal activities (Nóbrega *et al*., 2016). Initially, 0.09 mL of inoculum (with microbial suspension) was added to 2.91 mL of Sabouraud broth and incubated at 37°C. To this, a Sabouraud broth containing carvacrol or amphotericin B at each of the cited concentrations was added. After 0, 4, 8, and 24 hours, aliquots of 10 µL of each tube were uniformly sown in Petri dishes containing Sabouraud Dextrose Agar. The plates were incubated at 37°C for 24-48 hours. To compare the effect of the drugs (carvacrol and amphotericin B) in the test, the number of colony-forming units (CFU/mL) was summed at 24 hours in relation to the initial inoculum (time zero). The experiment was carried out in duplicate. When, as compared to the initial inoculum, a reduction was greater than 3 log10 or 99.9% in CFU/mL it was considered fungicidal (Klepser *et al*., 1998; Lewis *et al*., 2002).

Association Analyses

The time to death method was used to study the antimicrobial activity of the phytoconstituent against *C. neoformans*. The antifungal activity of each association of carvacrol, in concentrations of [81 µg/mL (MIC), 41 µg/mL (MIC/2), and 162 µg/mL (2xMIC)], combined with each of the amphotericin B concentrations [1µg/mL (MIC), 0.5µg/mL (MIC/2), and 2 µg/mL (2xMIC)] was evaluated. The minimum inhibitory concentrations of carvacrol and amphotericin B against this strain of *C. neoformans* were previously determined by our research group (Nóbrega *et al*., 2016). Initially, 0.09 mL of inoculum with microbial suspension was added to 2.91 mL of Sabouraud broth in tubes for incubation at 37ºC. The test drug concentrations, carvacrol and amphotericin B were added (at the various combinations mentioned above), and at 0, 2, 4, 8, 24 hours post-incubation, a 10µL aliquot from each tube was uniformly inoculated into separate Petri dishes containing Sabouraud Dextrose Agar. The positive control consisted of Sabouraud broth added to the inoculum. The plates were incubated in an oven at 37°C for 24-48 hours (Klepser *et al*., 1998; Keele *et al*., 2001; Lewis *et al*., 2002; Liu *et al*., 2014).

Synergism was defined as, the difference between the micro-organism count (CFU/mL) when exposed to the phytoconstituent/antifungal agent association and the micro-organism count after exposure to the phytoconstituent for 24 hours (being the most active), being greater than or equal to 2 log10. When this difference was between 2 and 1 log10, the action was classified as additive. When the difference was less than 1 log10, it was considered indifferent. When there was an increase of greater than or equal to 2 log10 in the CFU/mL association count as compared to the independent CFU/mL value of the most active drug alone, antagonism was defined (White *et al*., 1996; Ernst *et al*., 1999; Johnson *et al*., 2004).

Effect of carvacrol on C. neoformans micromorphology

For *C. neoformans* morphological alterations we employed micro-cultivation techniques for yeast, using solid agar-corn flour medium in a moist chamber (Kurtzman, 1998) and taking as a basis, results obtained by our research group in a previous study (Nóbrega *et al*., 2016).

Agar-corn flour (1 mL) was deposited in the presence or absence of carvacrol or antifungal drugs (control), in inhibitory and sub-inhibitory concentrations, being bonded onto a sterile slide, and restrained on a support within a moist chamber. After solidification of the medium, the yeast was sown onto three (Mcdonald *et al*., 2012) parallel strips using a platinum loop. The strips were then covered with sterile slides. To avoid drying of the medium during the incubation period, we placed a 3x3 cm square of filter paper (soaked in 2 mL of distilled water) in the moist chamber. The chamber was then incubated for 48 hours at 37°C (Riddel, 1950; Kon *et al*., 2008).

After 48 hours, the coverslip was removed and placed on a new slide, and a drop of india ink was added for visualization of the fungal capsule. The slides were then analyzed in an optical microscope at 400x magnification. To measure the size of the capsule, the ImageJ program was used evaluating 30 cells in each concentration tested, where the size of the cell wall of the capsule was considered. Measurements were performed in four different regions of the cell and an average obtained (Cardoso *et al*., 2016).

Statistical analysis

The software GraphPad Prism 8 was used. The results of micromorphology assay are expressed as mean ± standard error. They were analyzed using the Student t - test and Analysis of Variance (ANOVA) statistical tests, followed by Dunnett 's test.

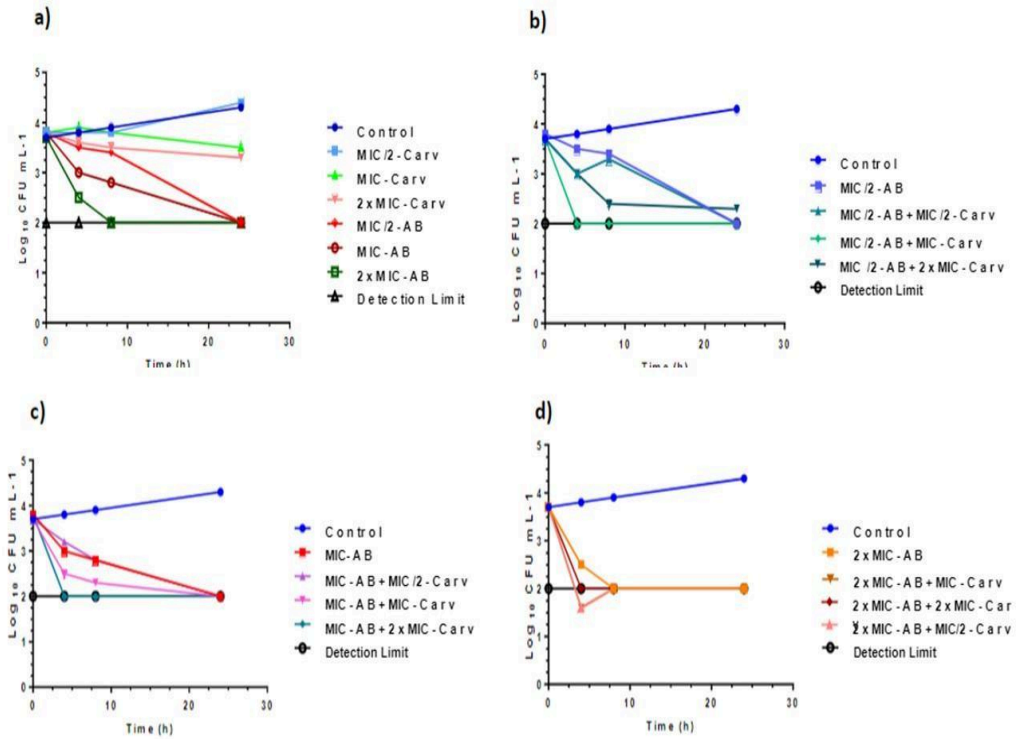
RESULTS AND DISCUSSION

In accordance with our results (Figure 1a), carvacrol, when tested alone at concentrations of 81 µg/mL (MIC), and 162 µg/mL (2xMIC), presents a fungistatic effect (a decrease of less than 99.9% (in CFU/mL) from the initial inoculum. This corresponds to a reduction of less than 3 log10 (CFU/mL). Amphotericin B, in all concentrations, presented a fungicidal effect quickly, in about 2 to 4 hours, with a greater than 99.9% (CFU/mL) decrease from the initial inoculum.

After the behavior of the phytoconstituent in growth kinetics modulation was evaluated, the next step was to investigate possible synergism in associations of carvacrol with amphotericin B. The results obtained in the carvacrol and amphotericin

B association studies using *Time to kill* testing are presented in figures b, c and d. These figures demonstrate neither synergism nor antagonism for the associations.

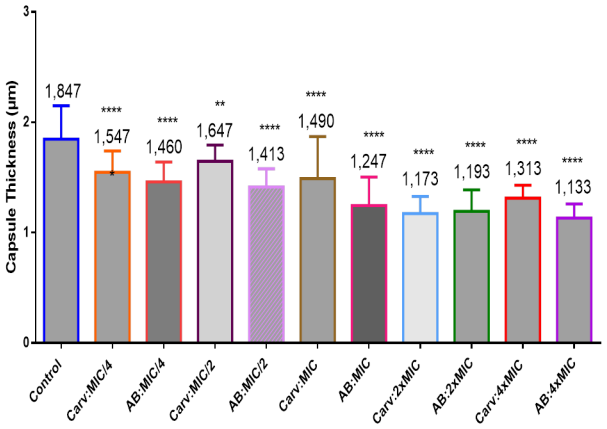
Figure 1 - Time to death of microbial cells treated with different concentrations of amphotericin B and carvacrol.



(A) Time to death from microbial amphotericin B in the minimum inhibitory concentration of 1 µg/mL (MIC), sub-inhibitory 0.5 µg/mL (MIC/2) and 2 µg/mL (2xMIC) and carvacrol in minimum inhibitory concentration of 81 µg/mL (MIC), sub-inhibitory 40.5 µg/mL (MIC/2) and higher 162 µg/mL (2xMIC) against *C. neoformans* (LM-22); **(B)** Time to death of the association of amphotericin B 0.5 µg/mL (MIC/2) in association with carvacrol in different concentrations 81 µg/mL (MIC), 41 µg/mL (MIC/2) and 162 µg/mL (2xMIC) against *C. neoformans* (LM-22); **(C)** Time to death for the association of amphotericin B 1 µg/mL (MIC) in association with carvacrol in different concentrations 81 µg/mL (MIC), 41 µg/mL (MIC/2) and 162 µg/mL (2xMIC) against *C. neoformans* (LM-22); **(D)** Time to death for association of amphotericin B 2 µg/mL (2xMIC) in association with carvacrol in different concentrations 81 µg/mL (MIC), 41 µg/mL (MIC/2) and 162 µg/mL (2xMIC) against *C. neoformans* (LM-22). AB= amphotericin B; Carv= carvacrol.

The capsule is an important virulence factor for *Cryptococcus*. Thus, using the microculture technique, both carvacrol at its MIC concentration, 2xMIC and 4xMIC, and at its sub-inhibitory concentrations of MIC/2 and MIC/4, and amphotericin (being the positive control) at the same concentrations, were tested against *C. neoformans* LM-22 to evaluate if these drugs interfere with capsule thickness. It was observed that both carvacrol and amphotericin B, at all concentrations, achieve significant reductions in capsule thickness compared to the control (figure 2).

Figure 2 - Effect of the Minimum Inhibitory Concentration (MIC) of amphotericin B and carvacrol on the capsule thickness of *Cryptococcus neoformans*.



Effect of MIC (81 µg/mL) and their concentrations above (2xMIC and 4xMIC) and sub-inhibitory (MIC/2 and MIC/4) of carvacrol and MIC (1 µg/mL), and their concentrations above (2xMIC and 4xMIC), and sub-inhibitory (MIC/2 and MIC/4) of amphotericin B about the thickness of the capsule. Values (expressed as means) were obtained by ANOVA with significance $p<0.05$ followed by Dunnett 's test. MIC= Minimum Inhibitory Concentration. AB= amphotericin B. Carv= carvacrol.

The ability of the inhibitory and sub-inhibitory concentrations of carvacrol and amphotericin B to reduce capsule thickness was compared, as shown in table 1. No significant difference in yeast capsule thickness was observed at the MIC/4 concentration for either drug.

Table 1 - Comparison of inhibitory and sub-inhibitory concentrations of carvacrol and amphotericin B on the size of the capsule of *C. neoformans* as seen through an optical microscope.

***Cryptococcus neoformans*: Strain: LM-22**	**Carvacrol[*] (µg/mL)**	**Amphotericin B[+] (µg/mL)**	**Capsule thickness measurement differences (µm)**	***p* Value**
	MIC	MIC	1,490 ± 0,06954[*] 1,247 ± 0,04691[+]	0,0053
	MIC/2	MIC/2	1,647 ± 0,02701[*] 1,413 ± 0,03022[+]	<0,0001
	MIC/4	MIC/4	1,547 ± 0,03515[*] 1,460 ± 0,03308[+]	NS

Values obtained by the Student's *t-test*. $p<0.05$: significant; NS: not significant; [*] = Carvacrol; [+] = amphotericin B.

With regard to antimicrobial action, a drug may present fungistatic and/or fungicidal action. Fungicidal action occurs when the drug achieves a reduction of greater than 99.9% in CFU/mL as compared with the initial inoculum. Carvacrol thus presents fungistatic activity at concentrations of 81 µg/mL and 162 µg/mL (figure 1), which is an interesting finding, as the treatment of cryptococcosis may involve fungistatic drugs such as azoles (Spadari *et al*., 2020).

No single method is considered the gold standard for assessing associations of antimicrobial agents (Johnson, 2004), the testing of carvacrol with amphotericin B was performed using microbial death kinetics. Indifference is defined as the combined effect of the drugs being similar to the effect of the more active drug alone (Cuenca-Estrella, 2004). Against *C. neoformans,* the association of carvacrol with amphotericin B presented as indifferent.

Pharmacological indifference is an interesting finding, as it allows the drug to be used associated with others without causing antagonism and reduced efficacy (Andrade Júnior *et al*., 2023).

Under the conditions evaluated, the monoterpene carvacrol was verified as reducing the thickness of the *C. neoformans* capsule, as compared to the control (Table 1). The results corroborate work carried out by Cardoso *et al*. (2016), in which linalool and geraniol also reduced the thickness of the *C. neoformans* capsule. Further, despite being a preliminary study, the ability of carvacrol to reduce the thickness of the *C. neoformans* capsule is relevant, since the capsule is an important virulence factor, assuring the survival of the yeast inside the affected individual.

As demonstrated in this study, the monoterpene carvacrol reduces capsule thickness. During infections this may contribute to an improved immune response since in vivo, large cryptococcosis cells affect phagocytosis negatively (Janbon, 2004). This has been demonstrated in another study, in which the macrophages typically phagocytized cryptococcosis cells with smaller polysaccharide capsules (Bojarczuk *et al*., 2016).

The mechanism by which amphotericin B affects the size of the capsule is still unknown. Many factors can influence the size of a capsule, such as osmotic pressure, nutrition, environmental factors, and the type of tissue infected. The presence of large capsules has been reported in the lung, but it is believed that the carbohydrates present and/or biosynthetic pathway reactions involved in the capsule are the principal targets for investigation (Zaragosa *et al*., 2009; Srikanta *et al*., 2014) However, modulation of capsule thickness may well be related to alterations involving individual molecules of glucuronoxylomannan, a polysaccharide (GXM) (Yoneda and Doering, 2008).

CONCLUSIONS

As observed in the Time to kill studies, when carvacrol was associated with amphotericin B, it showed pharmacological indifference, which is a positive finding, as this does not invalidate the use, in the future, of monoterpene, as a drug associated with other drugs licensed. Moreover, it is evident that the natural product allowed a significant reduction in capsule thickness, an important virulence factor present in the yeast *C. neoformans*.

Acknowledgements

The authors acknowledge CAPES for granting the scholarship to conduct this research.

References

AHN, K. The worldwide trend of using botanical drugs and strategies for developing global drugs. **BMB reports**, v. 50, n. 3, p. 111, 2017.

ANDRADE JÚNIOR, F. P. et al. Antifungal activity of citronellal against Trichophyton rubrum and its predictive mechanism of action by CYP51 inhibition through molecular docking. **Natural Product Research**, p. 1-9, 2023.

BOJARCZUK, Aleksandra et al. Cryptococcus neoformans intracellular proliferation and capsule size determines early macrophage control of infection. **Scientific reports**, v. 6, n. 1, p. 21489, 2016.

CARDOSO, N.N.R et al. Synergism effect of the essential oil from Ocimum basilicum var. Maria Bonita and its major components with fluconazole and its influence on ergosterol biosynthesis. **Evidence‐Based Complementary and Alternative Medicine**, v. 2016, n. 1, p. 5647182, 2016.

CLEELAND, R.; SQUIRES, E. **Evaluation of new antimicrobials "in vitro" and in experimental animal infections**. In: Lorian V, editor. Antibiotics in Laboratory Medicine. 3rd ed. New York: The Williams & Wilkins; 1991. p. 739-88.

CUENCA-ESTRELLA, Manuel. Combinations of antifungal agents in therapy–what value are they?. **Journal of antimicrobial chemotherapy**, v. 54, n. 5, p. 854-869, 2004.

ERNST, Erika J. et al. In vitro pharmacodynamic properties of MK-0991 determined by time-kill methods. **Diagnostic microbiology and infectious disease**, v. 33, n. 2, p. 75-80, 1999.

JANBON, Guilhem. Cryptococcus neoformans capsule biosynthesis and regulation. FEMS yeast research, v. 4, n. 8, p. 765-771, 2004.

JOHNSON, M. D. et al. Combination antifungal therapy. **Antimicrobial agents and chemotherapy**, v. 48, n. 3, p. 693-715, 2004.

KEELE, D. J. et al. Evaluation of amphotericin B and flucytosine in combination against Candida albicans and Cryptococcus neoformans using time-kill methodology. **Diagnostic microbiology and infectious disease**, v. 41, n. 3, p. 121-126, 2001.

KHAN, M. et al. Chemical diversity in leaf and stem essential oils of Origanum vulgare L. and their effects on microbicidal activities. **AMB express**, v. 9, p. 1-15, 2019.

KLEPSER, M. E. et al. Influence of test conditions on antifungal time-kill curve results: proposal for standardized methods. **Antimicrobial agents and chemotherapy**, v. 42, n. 5, p. 1207-1212, 1998.

KON, A.S. et al. Consensus in cryptococcosis - 2008. **Revista da Sociedade Brasileira de Medicina Tropical**, v.41,n.5, p.524-44, 2008.

KURTZMAN, C.P.; FELL, J. W. **The yeasts - A taxonomic study**. 4th ed. Amsterdam: Elsevier Science; 1998.

LEWIS, R. E. et al. Comparison of Etest, chequerboard dilution and time–kill studies for the detection of synergy or antagonism between antifungal agents tested against Candida species. **Journal of Antimicrobial Chemotherapy**, v. 49, n. 2, p. 345-351, 2002.

LIRA, M. *H.* P. *et al.* Antimicrobial activity of geraniol: An integrative review. **Journal of Essential Oil Research,** v. 32, n. 3, p. 187-197, 2020.

LIU, W. et al. Synergistic antifungal effect of glabridin and fluconazole. **PloS one**, v. 9, n. 7, p. e103442, 2014.

MCDONALD, T.; WIESNER, D. L.; NIELSEN, K. Cryptococcus. **Current Biology**, v. 22, n. 14, p. R554-R555, 2012.

NÓBREGA, R. O. et al. Investigation of the antifungal activity of carvacrol against strains of Cryptococcus neoformans. **Pharmaceutical biology**, v. 54, n. 11, p. 2591-2596, 2016.

RATHORE, S. S. et al. A holistic review on Cryptococcus neoformans. **Microbial pathogenesis**, v. 166, p. 105521, 2022.

RIDDELL, Roland W. Permanent stained mycological preparations obtained by slide culture. **Mycologia**, v. 42, n. 2, p. 265-270, 1950.

SOARES, E. S. et al. Mortality by cryptococcosis in Brazil from 2000 to 2012: A descriptive epidemiological study. **PLoS neglected tropical diseases**, v. 13, n. 7, p. e0007569, 2019.

SPADARI, C. C. et al. New approaches for cryptococcosis treatment. **Microorganisms**, v. 8, n. 4, p. 613, 2020.

SRIKANTA, D.; SANTIAGO-DRAWN, F.H.; DOERING, T.L. Cryptococcus neoformans: historical curiosity to modern pathos. **Yeast**, v.31, n.2, p.47-60, 2014.

WHITE, R. L. et al. Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. **Antimicrobial agents and chemotherapy**, v. 40, n. 8, p. 1914-1918, 1996.

YONEDA, A.; DOERING, T. L. Regulation of Cryptococcus neoformans capsule size is mediated at the polymer level. **Eukaryotic cell**, v. 7, n. 3, p. 546-549, 2008.
ZARAGOZA, O. et al. The capsule of the fungal pathogen Cryptococcus neoformans. **Advances in applied microbiology**, v. 68, p. 133-216, 2009.