International Journal of
Agriculture, Environment and Food Sciences
e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.2.7 Int. J. Agric. Environ. Food Sci. 2025; 9 (2): 325-330

Anti-infective effect of *Aquilaria malaccensis* L. essential oil against *Candida* strains, the leading cause of yeast infectious

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Article History
Received: April 01, 2025
Accepted: May 12, 2025
Published Online: June 15, 2025

Article Info
Type: Research Article
Subject: Medicinal and Aromatic Plants

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Available at
https://dergipark.org.tr/jaefs/issue/91914/1668986

DergiPark AKADEMİK

Abstract

Aquilaria malaccensis L., known as Agarwood, is widely found in India, Malaysia, Bhutan, and Indonesia. It is a pleasantly scented plant used in the production of resin. It is an interesting material in the field of health due to its resin and essential oil, which exhibit antimicrobial properties. This study aimed to evaluate the antifungal properties of *A. malaccensis* L. essential oil and determine its minimum inhibitory concentrations (MIC) against the *Candida* species tested. The inhibitory effect of *A. malaccensis* L. essential oil was tested on five *Candida* species. The broth microdilution method was used to determine the MIC values against the tested microorganisms, and the viability of microorganisms exposed to the plant essential oil was assessed using resazurin sodium salt. According to the results, the MIC of the plant essential oil against *Candida tropicalis, Candida krusei* and *Candida albicans* is 62.50 µL/mL. While the MIC against *Candida glabrata* is 31.25 µL/mL, the MIC against *Candida parapsilosis* is 7.81 µL/mL. These results show the potential of *A. malaccensis* L. as an anti-candidal agent. The continuation of this study revealed the need for optimizing and implementing more comprehensive antimicrobial tests.

Keywords: *Aquilaria malaccensis* L., Agarwood, Anti-candidal activity, Candidiasis, MIC value

Cite this article as: Unver, T., Erenler, A.S., Kiran, T.R. (2025). Anti-infective effect of *Aquilaria malaccensis* L. essential oil against *Candida* strains, the leading cause of yeast infectious. International Journal of Agriculture, Environment and Food Sciences, 9 (2): 325-330. https://doi.org/10.31015/2025.2.7

INTRODUCTION

Aquilaria malaccensis L., also known as Agarwood, is a plant from the Thymelaeaceae family with a long history. It is used in cultural rituals and medicine, especially in Asia. Agarwood is used in perfumery, cosmetics, medicines, balms, soaps and body lotions. It is known as gaharu, a precious tree, or a short tree in Indonesia. It is used in the production of perfume and incense due to the smell and structure of its resin (Naef, 2011). The composition of agar tree resin is mainly composed of mixtures of sesquiterpenes and 2-(2-phenylethyl) chromones (PECs) (Chen et al., 2012; Subasinghe et al., 2015). It has been shown that sesquiterpenoids and chromones are also present in its essential oil (Fazila et al., 2012; Jayachandran et al., 2014). Chromones are a large group of secondary metabolites with a wide range of potential therapeutic indications for immunomodulation, inflammation, cancer, diabetes, neurological conditions, and bacterial and viral infections (Tawfik et al., 2014). *A. malaccensis* L. tissues contain flavonoids, glycosides, triterpenoids, and tannins. The antimicrobial properties of these components are well-documented, and this effect has been demonstrated in studies published in the literature (Batubara et al., 2021). All these important compounds contribute to the antimicrobial activity of agarwood. It is an interesting material in the field of health because its resin and oil show antimicrobial properties (Yang et al., 2012; Gogoi et al., 2023). Its antimicrobial activity offers us the opportunity to discuss this plant and its content in terms of health. There is limited literature on its anti-candidal properties. Candidiasis is an infection caused by the overgrowth of *Candida* species in the body. It is frequently encountered in the oral mucosa and female reproductive system in babies. Due to regional sensitivities, natural ingredients are preferred in the treatment of

candidiasis. In our research, the anti-candidal property of *A. malaccensis* L. extract was tested using *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida albicans* and *Candida parapsilosis*. In previous studies, the genotoxicity, antioxidant, anti-inflammatory, anti-diabetic, acetylcholinesterase inhibitor, anti-urolytic, tyrosinase inhibitor, and antimicrobial properties of *A. malaccensis* essential oil have been investigated (Gogoi et al., 2023). There is limited data on its anti-candidal activity. Our research investigated the anti-candidal potential of *A. malaccensis* L. essential oil.

MATERIALS AND METHODS

Aquilaria malaccensis L. essential oil

In this research, Agarwood oil (Katre-i Sifa, Istanbul, Turkiye), a 100% natural, undiluted fragrance therapeutic grade essential oil for aromatherapy, was used. The essential oil was dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany) at a 1:1 ratio and subjected to antifungal testing.

Determination of Antifungal Activity

Microorganisms and Media

The antifungal activity of *A. malaccensis* L. essential oil was tested against *Candida albicans* and non-*albicans Candida* strains. Five *Candida* strains, the leading causes of *Candida* infections, were used in the activity tests. These strains are *Candida tropicalis* (ATCC 13803), *Candida glabrata* (ATCC 2001), *Candida krusei* (ATCC 14243), *Candida albicans* (ATCC 14053) and *Candida parapsilosis* (ATCC 22019), all purchased from the American Type Culture Collection (VA, USA). Sabouraud broth (Biolife, Milan, Italy) and sabouraud 4% glucose agar (Chemsolute, Renningen, Germany) were used for subculture and activity tests of *Candida* strains.

Determination of Minimum Inhibitory Concentration (MIC) for Yeast Strains

The minimum inhibitory concentration (MIC) of the tested oil was determined by making minor modifications to the previously applied method (Unver et al., 2024; Unver & Gurhan, 2024a and 2024b). Two dilution methods were applied for the antifungal activity determination test: agar and resazurin-based broth dilutions. In the broth dilution method, the essential oil, dissolved in DMSO, was diluted from the first to the tenth well on the microplate. Since 500 µL/mL of the oil added to the first well was diluted two-fold, it was present at 0.977 µL/mL in the tenth well. The eleventh and twelfth wells were negative and positive controls, respectively. The eleventh well represents the viability of the yeast strain used in the same row and contains only the broth medium and the yeast strain of the same row. The twelfth-row controller indicates no contamination when the experimental protocol was implemented and contains only broth medium. After diluting the essential oil, the standard inoculum of each strain was prepared. Each strain was suspended in distilled water, and the turbidity was adjusted to 0.5 McFarland (1-1.5 x 10^6 cfu/mL). Subsequently, 1 µL of each *Candida* suspension was inoculated into all wells except the negative control wells. *C. tropicalis, C. glabrata, C. krusei, C. albicans* and *C. parapsilosis* were inoculated into the wells in rows A, B, C, D and E, respectively. Then, the microplate was incubated at 35 °C for 24 hours. The next day, 15 µL of resazurin (0.15% v/v) (Sigma, Germany) was added to each well, and the microplate was incubated at 35 °C for 4-6 hours to observe the color change.

In the agar dilution method, essential oil was added to the first plate at a concentration of 500 µL/mL, and a two-fold dilution was made, starting from the first plate and proceeding to the eleventh plate. Therefore, the oil concentration varied between 500 and 0.488 µL/mL from the first plate to the eleventh plate. Afterward, as applied in the broth dilution method, a standard inoculum was prepared and inoculated onto the agar plates divided into five regions *C. tropicalis, C. glabrata, C. krusei, C. albicans* and *C. parapsilosis* were inoculated in the regions A, B, C, D and E on the plates, respectively. Subsequently, plates were left in an incubator and waited at 35 °C for 24 hours. The next day, MIC values were determined by observing the growth of colonies on the plates..

RESULTS AND DISCUSSION

In the broth dilution method, the color change on the microplate, from blue-purple to pink-salmon, indicates the active reproduction of the microorganism in the wells (Elshikh et al., 2016). Accordingly, *C. tropicalis, C. krusei,* and *C. albicans* inoculated in rows A, C, and D grew from the fifth well. For these reasons, the value in the well with the lowest oil concentration in which no growth was observed (62.5 µL/mL) was determined as the MIC value. *C. glabrata* inoculated into row B showed growth starting from the sixth well. Therefore, the MIC value for *C. glabrata* was determined to be 31.25 µg/mL. Finally, *C. parapsilosis* inoculated in row E grew from the eighth well. Thus, the MIC value for this microorganism was found to be 7.81 µL/mL (Figure 1).

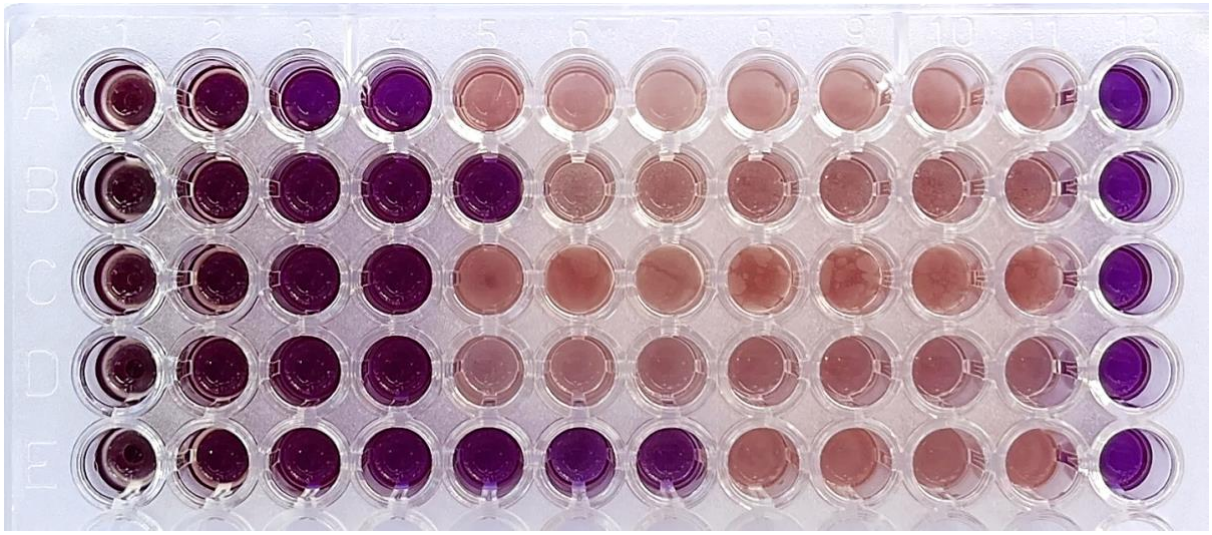

Figure 1. The antifungal activity of *Aquilaria malaccensis* L. essential oil against *Candida tropicalis* (A), *Candida glabrata* (B), *Candida krusei* (C), *Candida albicans* (D) and *Candida parapsilosis* (E) using the resazurin-based broth dilution method. The essential oil concentration ranged between 500 and 0.977 µL/mL from the first to the tenth wells. The eleventh and twelfth wells are negative and positive controls, respectively.

The agar dilution results were the same as the resazurin-based broth dilution results, and the MIC values were the same (Figures 2 and 3). As a result of the agar dilution method, *C. tropicalis, C. krusei,* and *C. albicans* inoculated into the first, third, and fourth zones on the agar plates started to grow from the E plate. Therefore, the concentration of oil in plate D (62.5 µL/mL) was determined as the MIC value. Since the colonies of *C. glabrata* inoculated to the second area on the plate started to appear from the F plate, the MIC value was determined as 31.25 µL/mL, which is the value in the plate with the lowest oil concentration in which no growth was observed. Finally, *C. parapsilosis* inoculated into the fifth zone showed growth starting from the H plate. Therefore, the MIC value of the oil against *C. parapsilosis* was determined to be 7.81 µg/mL (Figure 2).

Agarwood is used in Chinese medicine as a natural sedative, pain reliever, digestive aid, and carminative (Ye et al., 2016; Ma et al., 2021). *A. malaccensis* L. is a quality, fragrant, and medicinally significant plant-based essential oil with pharmacological potential and genotoxicity (Samadi et al., 2016). According to the results of our research, the anti-candidal activity of *A. malaccensis* L. was observed. The MIC value, which represents the minimum concentration of the essential oil required to inhibit the growth of the fungi, of *A. malaccensis* L. against *C. tropicalis, C. krusei* and *C. albicans* is 62.50 µL/mL. The MIC value against *C. glabrata* is 31.25 µL/mL, while the MIC value against *C. parapsilosis* is 7.81 µL/mL. These results support the anti-candidal activity of *A. malaccensis* L.

The dominant compounds in plant essential oil directly affect the medicinal and pharmaceutical use of the plant (Gogoi et al., 2023; Unver & Gurhan, 2024). In a study in the literature, the three most abundant compounds in the plant essential oil content were determined to be cubanol (22.26%), agarospirol (14.35%), and aristolene (13.22%) (Gogoi et al., 2023). Although antibacterial activity was tested in the same study, the disk diffusion method was employed, and the inhibition zones obtained were evaluated to draw inferences about the antimicrobial activity. Therefore, these results did not provide reliable MIC values (Gogoi et al., 2023). With their potential, these compounds are thought to contribute to the essential oil's antifungal activity, offering hope for future treatments.

It is crucial to develop this subject further for future research, particularly by applying different analyses at varying concentrations. The use of herbal agents in treating *Candida* species, which cause complex and persistent infections, is particularly necessary for women, mothers, and babies. *A. malaccensis* L. could serve as a potent alternative in this regard. While our research was not conducted under *in vivo* conditions, the preliminary nature of this study should be seen as an advantage in revealing the potential of *A. malaccensis* L. as a natural anti-candidal agent. This underscores the importance of continued research in this area.

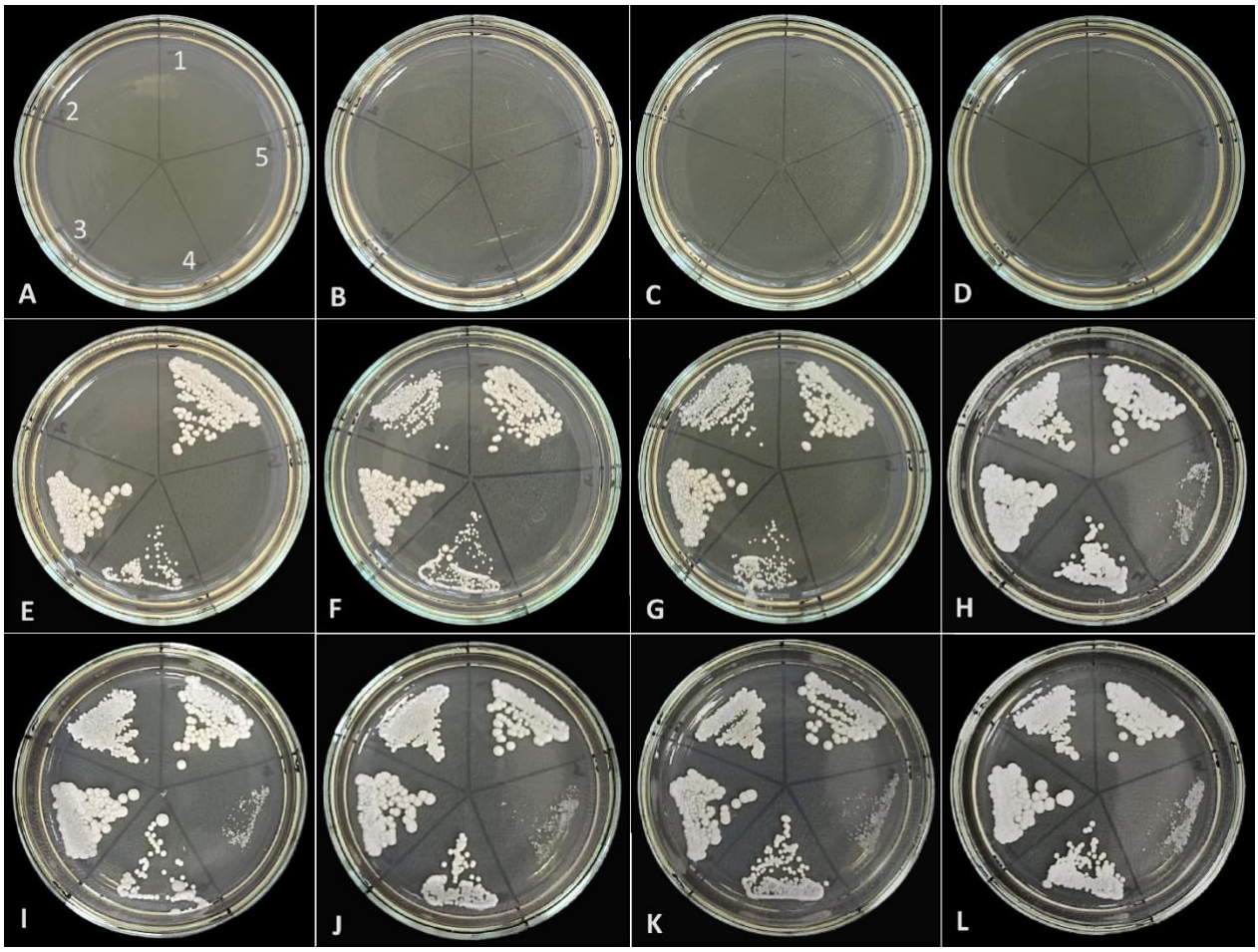

Figure 2. The antifungal activity of *Aquilaria malaccensis* L. essential oil against *Candida tropicalis* (1), *Candida glabrata* (2), *Candida krusei* (3), *Candida albicans* (4) and *Candida parapsilosis* (5) using agar dilution method. The essential oil concentration ranged from 500 to 0.488 µL/mL across plates A to K. The twelfth plate (L) is the control.

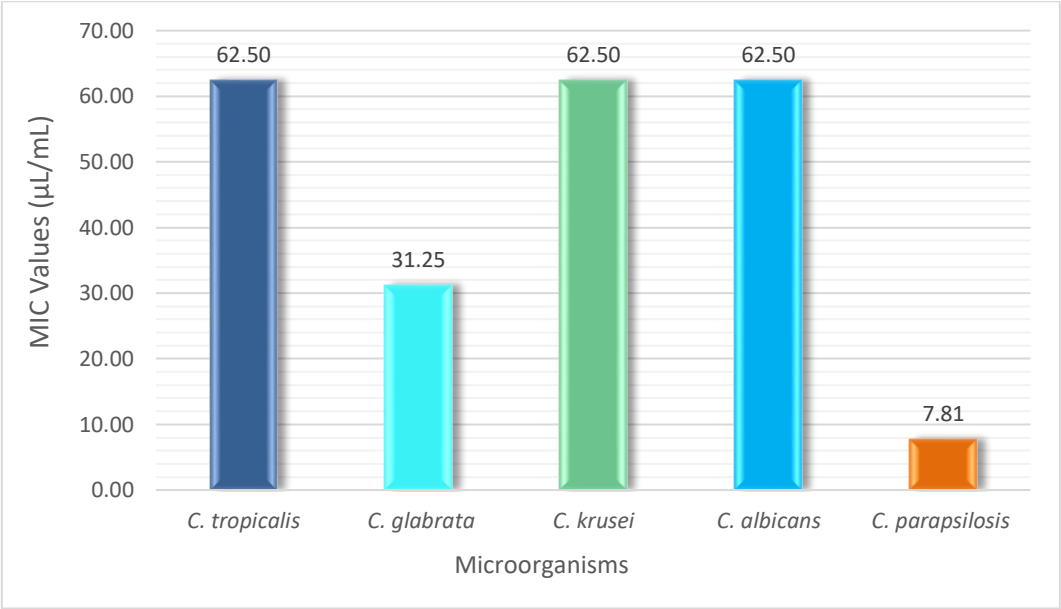

Figure 3. Comparative MIC values of *Aquilaria malaccensis* L. essential oil against tested yeast species

CONCLUSION

A. malaccensis L. is a natural and reliable therapeutic agent with antimicrobial properties. Studies conducted with Agarwood essential oil in the literature to date have indicated the existence of important pharmacological properties of this oil (Zhang et al., 2025; Ma et al., 2025; Jayaprakash et al., 2025). Therefore, scientists have always been interested in studies on Agarwood. Its potential for use in infections that are both dominant and difficult to treat, such as *Candida* infections, has been studied to a limited extent in the literature. This preliminary study has raised the possibility that *A. malaccensis* L. essential oil could be used for *Candida* infections. In subsequent studies, the necessity of interspecies comparisons and different analyses was revealed.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflicts of interest.

Author contribution

All authors declare that they have participated in the design, execution, and analysis of the study and have approved the final version.

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