

Original Research Report

ANTIFUNGAL ACTIVITY OF ENDOPHYTIC BACTERIA ISOLATED FROM MIANA PLANTS (*Coleus scutellarioides* (L.) Benth.) AGAINST *Candida albicans*Melda Yunita*^{ID}, Ruth Magdalena Lumbantobing, Ritha Tahitu^{ID}

Department of Medical Education, Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia

ABSTRACT

Candida albicans is the most common organism responsible for both mucosal and systemic infections, accounting for approximately 70% of fungal infections worldwide. Miana, scientifically known as *Coleus scutellarioides* (L.) Benth., is recognized for its use in traditional medicinal practices. Miana plants contain endophytic bacteria that possess the ability to produce secondary metabolites with potential antifungal agents. The objective of this study was to assess the antifungal activity of nine endophytic bacteria isolates derived from *Coleus scutellarioides* against *Candida albicans*. This study was laboratory-based qualitative experimental research that applied the Kirby-Bauer diffusion method and several modifications. The *Candida albicans* specimens were spread throughout the entire potato dextrose agar medium. Afterward, paper discs that had been soaked in a liquid culture of endophytic bacterial isolates were carefully placed on the surface of the medium. The complete setup was then incubated for 1–2 days. The potential antifungal activity of endophytic bacteria was assessed by observing the emergence of a clear zone surrounding their growth, which would indicate inhibition. An additional observation was performed in the follow-up test, involving the use of Sabouraud dextrose agar medium to confirm the initial test result. The results from the inhibitory test revealed that none of the bacterial isolates exhibited any inhibition zone. Conversely, ketoconazole as the positive control showed an inhibition zone with an average diameter of 28.5 mm. In conclusion, endophytic bacterial isolates obtained from *Coleus scutellarioides* have no discernible antifungal properties against *Candida albicans*. This study implies that ketoconazole remains effective in treating infections caused by *Candida albicans*.

Keywords: *Candida albicans*; *Coleus scutellarioides* (L.) Benth.; endophytic bacteria; human and health; tropical disease

*Correspondence: Melda Yunita, Department of Medical Education, Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia. Email: meldayunita22@gmail.com

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Highlights:

1. Due to the adverse effects associated with current antifungal drugs, research on traditional medicine is necessary to explore other options for candidiasis treatment.
2. Following the findings of this study, it is recommended to conduct further research by incorporating *Coleus scutellarioides* filtrate into the growth medium, since this may improve bacterial growth by producing optimal secondary metabolites.

INTRODUCTION

Candidiasis, an infection caused by *Candida*, is the most prevalent fungal infection in the world. Of all *Candida* species, *Candida albicans* is the most common cause of mucosal and systemic infections, accounting for around 70% of all fungal infections worldwide (Bhateja 2018, Talapko et al. 2021). Candidiasis is one of the most frequently found fungal infections in tropical countries, such as Indonesia. High temperatures and humidity may

contribute to this health issue (Makhfirah et al. 2020). *Candida* is a commensal fungus that typically lives on the skin and within several parts of the human body, including the mouth, throat, intestines, and vagina. This microbe is a normal flora when present in normal amounts. However, *Candida* may cause infection when it experiences uncontrolled growth or when the immune system of the host is compromised (Romo & Kumamoto 2020). In the presence of predisposing factors, *Candida albicans* can undergo a transition to become a pathogen,

resulting in the development of a candidiasis infection.

A study conducted between 2013 and 2016 at the Mycology Division of the Skin and Genital Health Outpatient Unit, Dr. Soetomo General Academic Hospital in Surabaya, Indonesia, revealed a rise in candidiasis cases. The study identified a yearly increase in the number of patients diagnosed with candidiasis, with a total of 99 patients (6.23%) in 2013, 77 patients (6.08%) in 2014, 55 patients (5.85%) in 2015, and 67 patients (8.97%) in 2016 (Puspitasari et al. 2019). In the study conducted by Maulana et al. (2019), it was observed that the prevalence of invasive candidiasis in the Intensive Care Unit at Dr. Hasan Sadikin Central General Hospital, Bandung, Indonesia, during the period of June 2016 to June 2017 was 3.5%. Furthermore, the investigation revealed an exceptionally high mortality rate of 81.8% associated with this condition. According to data provided by the Maluku Provincial Health Office, the number of candidiasis cases among individuals aged 15–24 years in Ambon City, Indonesia, showed fluctuations over the years. Specifically, there were 129 cases reported in 2014, followed by an increase to 196 cases in 2015, and a subsequent decrease to 129 cases in 2016 (Tidore 2018).

One of the drugs commonly used to overcome fungal infections is ketoconazole. However, ketoconazole is known to have side effects such as pruritus, nausea, rash, abdominal pain, headache, dizziness, fatigue, impotence, menstrual disorders, and gynecomastia (Lieberman & Curtis 2018, Sinawe & Casadesus 2023). Considering these side effects, people are increasingly opting for alternative treatments that incorporate natural ingredients or traditional medicinal practices. Miana is among the botanical species used in traditional medicinal practices. Scientifically known as *Coleus scutellarioides* (L.) Benth., miana is a native botanical species originating from India and Thailand. Miana plants reproduce vegetatively and are easy to find in various places. The plants are also endemic in Maluku, Indonesia, and are widely used by the local people to treat many different ailments, such as typhoid, cough, menstrual pain, boils, and fever (Wakhidah & Silalahi 2018).

It is noteworthy that multiple studies have demonstrated the presence of beneficial characteristics in these particular plants. The studies carried out by Muljono et al. (2016) and Setianingrum et al. (2014) observed and documented the antibacterial and antifungal activities of miana plants. Other studies have found evidence regarding the anti-inflammatory effects of the plants. It was reported that miana plants contain antioxidant and anti-diabetic compounds

(Marpaung 2014, Levita et al. 2016, Novanti & Susilawati 2017). Similar to other plant species, miana plants typically contains endophytic bacteria, which can produce secondary metabolites that resemble those of their host plants. Consequently, these endophytic bacteria have potential as antifungal agents (Yunita et al. 2022).

Endophytic bacteria refer to a group of microbes that colonize the tissues of healthy plants. However, the bacteria exist in a state of commensalism without eliciting any symptoms or inflicting harm on their hosts (Wu et al. 2021, Yunita et al. 2022). Endophytic bacteria are capable of producing secondary metabolites, including saponins, alkaloids, and terpenoids. In addition, they possess the ability to synthesize cell wall-degrading enzymes, such as chitinase, which exhibit antibiosis properties and are able to function as antifungal agents (Yunita et al. 2016, Vinayarani & Prakash 2018, Foeh et al. 2019). Several researchers have isolated a range of endophytes and conducted experiments to assess their abilities against *Candida albicans*. Rachman & Sari (2020) demonstrated the inhibitory effect of endophytic bacteria derived from *Lansea coromandelica* (Houtt.) Merr. on the growth of *Candida albicans*. Moreover, a recent study documented the presence of endophytic bacteria, classified as isolate K3, within the rhizome of *Curcuma longa*. The bacterial isolates showed high antioxidant properties, indicating their potential as a strong antimicrobial agent against *Candida albicans* (Sulistiyan et al. 2016). Nevertheless, there is currently a lack of research exploring the inhibitory potential of endophytic bacteria derived from miana plants (*Coleus scutellarioides*) against *Candida albicans*.

In a prior study conducted by Mahulette (2022), a total of nine endophytic bacterial isolates were obtained from the leaves, stems, and roots of miana plants. The isolates were assigned the codes A2, A3, A4, B1, B2, B4, D2, D3, and D4. The examined isolates have demonstrated the capacity to inhibit the growth of *Salmonella typhi*, as evidenced by the largest inhibition zone diameter of 27.5 mm, indicating a robust inhibitory effect. Given the findings of the previous investigation, which indicated the strong antibacterial activity of endophytic bacterial isolates obtained from miana plants, it is critical to identify and assess the antifungal capacity of these isolates as well. Hence, the primary objective of this study was to examine the inhibitory potential of endophytic bacteria obtained from miana plants against *Candida albicans*. This research offers an initial step towards the future development of these isolates as effective antifungal agents.

MATERIALS AND METHODS

The study was conducted from the 29th of December 2022 to the 4th of January 2023 at the Maluku Provincial Health Laboratory and Medical Device Calibration Center, Ambon, Indonesia. This study used a true experimental laboratory research design and an agar diffusion method. The ethical clearance was issued by the Department of Medical Education, Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia, with reference No. 173/FK-KOM.ETIK/VIII/2022 on 22/12/2022. This study was conducted as a continuation of the previous study by Mahulette (2022) on the potential inhibitory effect of bacterial isolates derived from miana plants against *Salmonella typhi*.

The main materials used in this study were nine endophytic bacterial isolates derived from miana plants (*Coleus scutellarioides* (L.) Benth.). These bacterial isolates were acquired from the previous study conducted by Mahulette (2022). The endophytic bacteria were initially re-cultured by transferring them onto a new nutrient agar medium produced by Merck KGaA (Darmstadt, Germany) with batch number 105540. Afterwards, the cultures were incubated at a temperature range of 28–30 °C for 24 hours (Jamilatun et al. 2020). Meanwhile, the pathogenic fungus used in this study was *Candida albicans* ATCC 90029, sourced from the Maluku Provincial Health Laboratory and Medical Device Calibration Center, Ambon, Indonesia (-3°69' 32.6"S, 128°19'82.4"E). The process of re-culturing *Candida albicans* involved the inoculation of the fungi on a petri dish using Sabouraud dextrose agar medium produced by Merck KGaA (Darmstadt, Germany) with batch number 146028. Subsequently, the petri dish was placed in an incubator set at a temperature of 37 °C for a duration of 24 hours (Zuraidah et al. 2021).

Analyses of *Candida albicans* were performed by including both macroscopic and microscopic observations of the fungus. The growth of the fungal culture in Sabouraud dextrose agar medium was assessed through macroscopic observations, which involved examining the color and surface characteristics of the colonies. *Candida albicans* was characterized by yeast-like colonies and a yellowish-white coloration. Additionally, the surface of the colonies was seen to be wet and convex in nature (Indrayati & Sari 2018). Microscopic observations were conducted after Lugol's solution staining. Then, the preparations were observed under a microscope with 100X magnification (Sulmiyati et al. 2019).

The inhibition test was carried out using the disc diffusion method. The suspension of *Candida albicans* ATCC 90029 was spread over Sabouraud

dextrose agar media using a sterilized swab. Blank paper discs were soaked in endophytic bacteria isolate suspension and a control solution (Sulistrioningsih et al. 2020). The experimental setup included the use of a ketoconazole solution with batch number 4591 as the positive control. Additionally, distilled water served as the negative control (Maulana et al. 2020). After a 24-hour incubation period, the measurement of the generated inhibition zone was conducted. The measurement of the inhibition zone was calculated using a defined formula that considered three key points: the vertical diameter (DV), horizontal diameter (DH), and paper disc diameter (DC) (Alioes & Kartika 2019, Winastri et al. 2020).

$$\frac{(DV - DC) + (DH - DC)}{2}$$

RESULTS

The macroscopic observations of *Candida albicans* ATCC 90029 after 48 hours of incubation revealed colonies with a yellowish-white coloration and round, moist, and convex surfaces (Figure 1). The microscopic observations of *Candida albicans* were conducted at 100X magnification. The findings from the observations indicated the presence of cells with an oval shape and a purple color. In addition, it was observed the presence of budding yeast cells, which exhibited asexual reproductive characteristics (Figure 2).

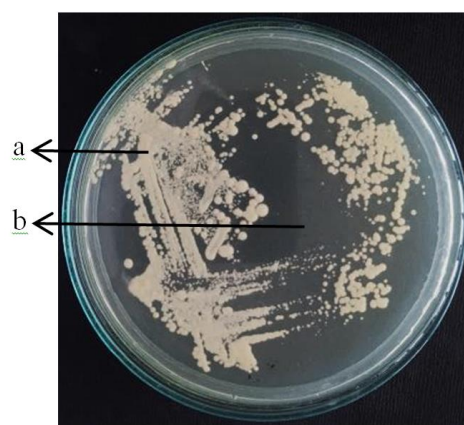


Figure 1. Macroscopic appearance of *Candida albicans* after 48 hours of incubation. a) *Candida albicans* colonies; b) Sabouraud dextrose agar media.

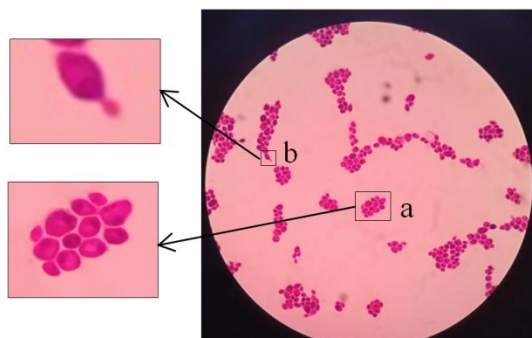


Figure 2. Microscopic observations of *Candida albicans*. a) *Candida albicans* colonies under the microscope; b) A single cell of *Candida albicans*.

The observation of the inhibition zone was conducted after incubating the petri dish for 48 hours. The results obtained from the observations indicated that the bacterial isolates tested did not exhibit any inhibition against *Candida albicans*. This was proven by the absence of a clear zone

around the disc paper containing endophytic bacteria in all replicates. The positive control, ketoconazole, had significant inhibitory activity in all replicates, as seen by inhibition zone diameters of 26 mm, 30 mm, and 29.5 mm. In contrast, the negative control, distilled water, failed to show any inhibition zone (Figure 3).

Upon the completion of the initial inhibition test, which yielded no observable inhibitory activity, a follow-up re-inhibition test was conducted with a different method. The bacterial diffusion method was employed with the aim of confirming the earlier findings. Instead of using paper discs, pure cultures of endophytic bacteria were taken using a sterile cotton swab. These cultures were then placed onto Sabouraud dextrose agar media that had been previously inoculated with *Candida albicans*. The data obtained after 48 hours of incubation indicated the same results as the initial inhibition test, where there was no clear zone around the endophytic bacteria.

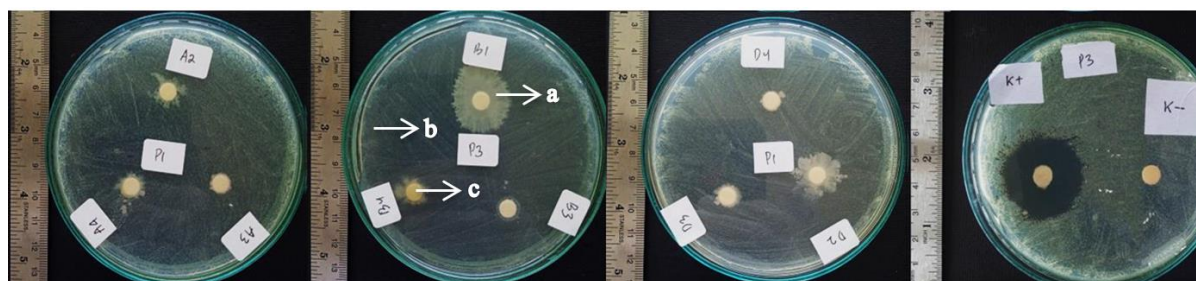


Figure 3. Results of inhibition test of nine endophytic bacterial isolates (isolates A1, A2, A3, B1, B2, B3, D1, D2, and D3) after 48 hours of incubation. a) Representation of endophytic bacteria; b) Population of *Candida albicans*; c) Disc paper used for the experiment. Notes: K(+): Ketoconazole as the positive control; K(-): Distilled water as the negative control.

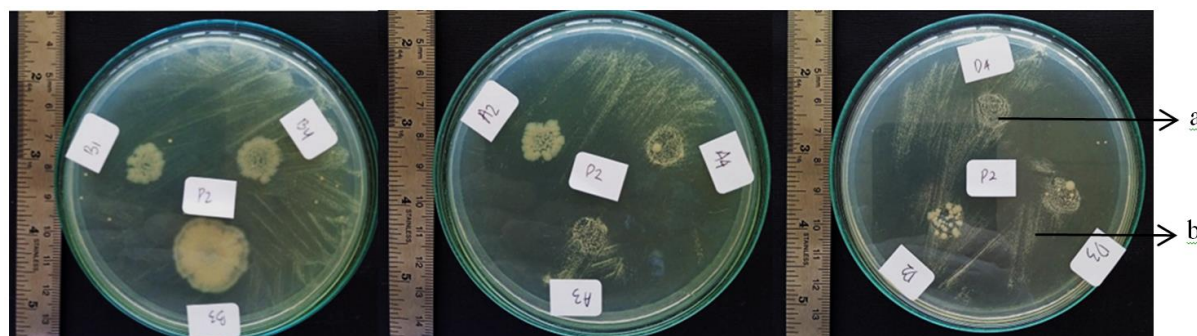


Figure 3. Re-inhibition test after 48 hours of incubation. a) Representation of endophytic bacteria; b) Population of *Candida albicans*.

DISCUSSION

The findings gathered from the tests and observations conducted in this study indicated that the bacterial isolates derived from miana plants were unable to inhibit the growth of *Candida albicans* ATCC 90029. Previous research conducted by Mahulette (2022) proved that endophytic bacteria derived from miana plants possess the capability to inhibit the growth of *Salmonella typhi*. However, it was discovered that the bacteria showed an inability to hinder the proliferation of *Candida albicans*. This might be attributed to the differences in the structure of bacteria and fungi. Fungi comprise both a cell wall and a plasma membrane, making them structurally thicker compared to bacteria, which only contain a cell wall. The cell wall of *Candida albicans* is characterized by its complex structure, with 100–400 nm in thickness. It is composed of five distinct layers stacked in an outer-to-inner arrangement. These layers include the fibrillar layer, mannoprotein, β -glucan, β -glucan-chitin, and mannoprotein (She et al. 2016). The many components of the fungal cell wall collaborate synergistically to maintain cellular integrity and prevent the infiltration of extracellular substances into the cell. *Candida albicans* possesses a plasma membrane consisting of ergosterol, a fungal sterol layer that plays an important part in modulating permeability and regulating the fluidity of membranes (Rubio et al. 2020). The complex structure of the cell wall and plasma membrane in *Candida albicans* might be the factor that contributed to the incapacity of endophytic bacteria's secondary metabolites in inhibiting the growth of *Candida albicans* in this study.

In a study conducted by Aprianti et al. (2019), the antifungal activity of red algae (*Eucheuma spinosum*) extract was examined against *Candida albicans* through phytochemical tests. Despite the presence of alkanoids, flavonoids, steroids, and triterpenoids in red algae, which have been shown to act as antifungals, no inhibitory effect was shown when these compounds were tested against *Candida albicans*. In contrast, a study conducted by Rachman & Sari (2020) proved that a total of 12 bacterial isolates derived from *Lannea coromandelica* were able to inhibit the growth of *Candida albicans*, with the largest inhibition zone diameter measured at 20.75 mm. In addition, a further investigation involved the culture of endophytic bacteria derived from plants of the *Zingiberaceae* family. These bacteria were tested against *Candida albicans*, which led to the observation of an inhibition zone with the largest diameter of 23.7 mm (Nursalwa et al. 2018).

The pathogenicity of *Candida* infections is attributed to various virulence factors, including

adhesins, virulence enzymes, and the morphological transition from yeast to hyphae. These characteristics are typically observed in *Candida* infections. In addition, infections caused by *Candida* spp. are distinguished by the formation of biofilms. Biofilms are structures that are embedded within the extracellular matrix (ECM) and have a complex three-dimensional architecture consisting primarily of yeast-shaped cells and hyphal cells with significant heterogeneity (Pierce et al. 2013). The formation of a biofilm starts with the attachment of fungal cells to the surface of the substrate. The biofilm then undergoes a series of stages, including proliferation, maturation, and expansion, which eventually result in the completion of the biofilm development. This cycle can repeat itself, leading to the further extension of the fungal population (Fanning & Mitchell 2012). Biofilm cells possess notable advantages in terms of survival compared to free-living cells since they exhibit enhanced resistance to various antimycotic treatments. The enhanced resistance of *Candida* biofilms to antimycotic drugs and their ability to counter immune defenses, along with their role as a persistent infection reservoir, have substantial clinical implications. The prevalence of biofilms significantly increases the morbidity and mortality associated with *Candida albicans* (Pierce et al. 2013). The formation of biofilms is widely recognized as a prominent virulence factor of *Candida albicans*. It is conceivable to suggest that endophytic bacterial isolates without evident antifungal activity may be incapable of limiting the growth of *Candida albicans*.

In another perspective, it is worth considering that there was a possible factor that could have influenced the results of this study. In a study conducted by Mahulette (2022), the researcher examined endophytic bacteria that were obtained from miana plants. However, the current research re-cultured nine endophytic bacterial isolates without incorporating any additional miana plant filtrate. Host plants and environmental factors play an important role in the formation of secondary metabolites by endophytic bacteria (Wu et al. 2021). In this study, the secondary metabolites produced by endophytic bacterial isolates might have decreased in quantity and quality. The absence of host plant activity, which could help the secretion of secondary metabolites, was a potential cause of this condition. An additional plausible factor was the environmental differences that existed between the host plant and the growth medium. A study conducted by Yunita et al. (2022) isolated endophytic bacteria obtained from *Myristica fragrans*. The results showed that the addition of host plant filtrate to the bacterial isolation process led to a higher total bacterial population, as the plant filtrate contains the growth factors of the endophytic

bacteria.

In the three repetitions of this study, ketoconazole demonstrated a high capacity to inhibit the growth of *Candida albicans*, as evidenced by inhibition zone diameters of 29.5 mm, 26 mm, and 30 mm. Ketoconazole acts as an antifungal agent by blocking the synthesis of ergosterol, thereby weakening the structure and function of the fungal cell membrane (Sinawe & Casadesus 2023). In a similar examination, Kalsum & Ayu (2019) used ketoconazole as a positive control in a test of *Candida albicans*. Their findings revealed inhibition zones with an average diameter of 27.1 mm, signifying a very strong inhibitory effect. As demonstrated by these results, ketoconazole is one of the antifungal drugs that remains sensitive to *Candida albicans*. However, taking into account the potential side effects, ketoconazole should only be administered when absolutely necessary and in accordance with the dosage prescribed by a physician.

Strength and limitations

Miana plants are frequently used as an alternative medicine throughout Indonesia, including the region of Maluku. *Candida albicans* had never been tested against endophytic bacteria derived from Miana plants (*Coleus scutellarioides*). Therefore, the results of this study are important for the development of antifungals using endophytic bacteria derived from miana plants. However, the results might be influenced by the fact that we did not add miana plant filtrate to the growth medium. The addition of host plant filtrate would affect the content of secondary metabolites produced by the endophytic bacteria.

CONCLUSION

Endophytic bacteria derived from miana plants (*Coleus scutellarioides* (L.) Benth.) lack the ability to inhibit the growth of *Candida albicans*. This study implies that ketoconazole remains effective in overcoming infections caused by *Candida albicans*. It is recommended for healthcare professionals who have prescriptive authority to use caution while administering antifungal medications to mitigate the risk of developing fungal resistance.

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Conflict of interest

None.

Ethical consideration

The Department of Medical Education, Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia, granted ethical clearance for this study with reference No. 173/FK-KOM.ETIK/VIII/2022 on 22/12/2022.

Funding disclosure

None.

Author contribution

All authors contributed to the research process, data analysis, interpretation of results, and drafting of the article. In detail, MY contributed to the research conception and design, critical revision of the article for important intellectual content, and final approval of the article. RML contributed to the analysis and interpretation of the data, as well as the collection and assembly of the data. Lastly, RT contributed to the critical revision of the article for important intellectual content and final approval of the article.

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