Mangrove Ethanol Extract (*Aegiceras corniculatum*) Failed to Inhibit *Candida albicans* Growth Isolated from Oral Candidiasis HIV/AIDS patient *in vitro*

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Abstract

Candida albicans species can cause severe morbidity and mortality in Human Immunodeficiency Virus (HIV) patients when they become systemically involved, known as candidemia. Mangrove (Aegiceras corniculatum) is a plant that is commonly distributed and has been shown to have several advantages to be used as phytotherapy.

Objective to investigate the anti-fungal ability of Mangrove Ethanol Extract (MEE) (A.corniculatum) to Candida albicans isolated from Oral Candidiasis HIV/AIDS patient in vitro.

The fresh mangrove leaf (A. corniculatum) was extracted by means of maceration methods. There were 5 groups, each with 4 samples: (1) control negative group (2) control doxycycline group, (3) MEE (A. corniculatum) concentration 25%, (3) 50% concentration group, (4) 75% concentration group, and (5) 100% concentration group. C. albicans isolated from stadium HIV/AIDS patients who were assessed clinically but did not seek fungal infection therapy. Saboraoud dextrose agar (SDA) containing the tested C. albicans suspension was poured into a petri dish around the paper disk. Observation of the inhibition zone around the well and then measuring the diameter of the inhibition zone horizontally and vertically using a digital scale ruler.

It was discovered that 100%, 75%, 50%, and 25% MEE (A corniculatum) had no antifungal efficacy against C. albicans isolated from HIV/AIDS with no significant difference in inhibitory zone ($p>0.05$).

MEE (A. corniculatum) did not have an inhibitory zone against C. albicans isolated from HIV/AIDS patients in vitro.

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Introduction

Acquired Immune Deficiency Syndrome (AIDS) is a group of symptoms produced by infection with the Human Immunodeficiency Virus (HIV), which has a life cycle in the human body. This virus spreads by sexual contact, bodily fluids, particularly blood (for example, during delivery, blood transfusions, and medication injection usage).[1] HIV/AIDS is now a severe disease that must be treated with extreme caution since it spreads rapidly, and it is also one of the top-ranking infectious diseases that can cause mortality. Four types of influences impact the degree of health in the environment. These factors include heredity, health-care services, behavioral issues, and environmental

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influences.[2] Heredity, health-care services, behavioral difficulties, and environmental effects are among these factors. HIV/AIDS is a form of infectious illness that falls under the category of sexually transmitted diseases. The most recent data on persons living with HIV/AIDS in the globe reached 33.3 million people. And this figure continues to rise year after year.[1,2] In 2015, Indonesia had the highest rate of HIV/AIDS infection in Southeast Asia, with 0.5 out of 1000 people affected. Meanwhile, it reached 0.3 out of 1000 people in other nations such as Myanmar, Malaysia, and Vietnam. Thailand and Laos have the highest rates, with 0.2 out of 1000 people, while the Philippines and Cambodia have the lowest rates, with 0.1 out of 1000 people.[3]

HIV/AIDS remains a global public health issue, especially in Indonesia. There are several factors that contribute to the high mortality rate in persons living with HIV, including not just viral infection but also opportunistic infections and other severe disorders.[4-6] Every year, the prevalence of HIV infection rises, which is directly associated to various parameters such as age, gender, risk factors for HIV infection, clinical stage of HIV, and type of HAART.[7,8]

The HIV/AIDS a condition that inhibits the sufferer's immune system, making various forms of infections that are simple to enter or diseases that the sufferer will suffer from possible.[9] The oral cavity is one of the few places where HIV may be transmitted mucosally. Many of the hazards of HIV/AIDS-related illness are linked to the patient's immunosuppression.[1]. Candidiasis is one of the illnesses caused by opportunistic infections and is now a condition that develops in persons with HIV/AIDS.[11-13] The current Candida infection spectrum ranges from asymptomatic colonization through Necrotizing Ulcerative Gingivitis or Periodontitis, Oropharyngeal Candidiasis (OPC), esophagitis, onychomycosis, vulovaginitis, cutaneous candidiasis, systemic candidiasis, and invasive candidiasis, including candidemia.[14,15]

During the course of the disease, one-third of HIV-infected individuals and 90% of AIDS patients acquire candidiasis. Patients with candidiasis frequently describe burning in the mouth, changes in taste, a bitter or salty flavor, and occasionally unpleasant discomfort, dysphagia, nausea, vomiting, and diarrhea. Symptoms such as eating disorders suggest a decline in the patient's quality of life.[16,17] In one research, 165 HIV-positive individuals were diagnosed with candidiasis, with the rest suffering from doropharyngeal candidiasis, oesophageal candidiasis, candidemia, pulmonary candidiasis, cutaneous candidiasis, and candida diarrhea. The majority of HIV patients (71.25 percent) had oral candidiasis, whereas the remainder had oesophageal candidiasis and candidemia.[18]

Oral Candidiasis is a fungus-caused opportunistic infectious illness. Candida is a typical part of the flora in healthy people. Candida is predicted to be present in 45–65 percent of healthy babies and 30–55 percent of healthy adults. Various systemic and other local causes can promote Candida species overgrowth on the oral mucosa, making oral candidiasis a serious concern in oral dermatology.[19] *Candida albicans* is the most common species seen in oral opportunistic infectious illnesses.[20] Although non-albicans such as C*andida tropicalis, Candida parapsilosis, Candida glabrata, Candida krusei*, and *Candida dubliniensis* have been discovered in the recent decade, Candida albicans remains the most prevalent species detected in oral candidiasis in HIV/AIDS patients. *C. albicans* overgrowth is caused by a reduction in the usual bacterial flora in the mouth, as well as other factors that allow HIV/AIDS to progress.[21,22]

Some *C. albicans* species can cause severe morbidity and death in HIV patients when they become systemically involved. *C. albicans*-related candidemia has a mortality rate of more than 30% in some groups.[23] Antifungal pharmacological treatment is administered to HIV/AIDS patients with opportunistic fungal infections. The treatment's goal is to prevent and selectively destroy pathogenic fungus in the host while causing minimum harm. Antifungal medications classified as polyenes (nystatin and amphotericin B), azole ergosterol biosynthesis inhibitors (miconazole, clotrimazole, ketoconazole, itraconazole, and fluconazole), and novel medicines such as caspofungin can be used to treat oral candidiasis. Cell membranes, cell walls, and nucleic acids are the primary targets of these antifungals.[24,25]

To anticipate the negative effects and resistance of this fungal treatment, an alternative medicine that may cure *C. albicans* infection with low side effects is required. Candidiasis is a prevalent illness in HIV patients because it is an opportunistic infectious disease. At some point

throughout their AIDS journey, over 90% of individuals had oral or oropharyngeal candidiasis.[26] Many investigations have been conducted in order to get medicinal substances as natural antifungals with extremely low levels of negative effects when evaluated *in vitro*. Berberine, for example, is one of the active compounds examined that has antifungal activity against Candida albicans. Berberine is a naturally occurring bioactive molecule found in a variety of plants, with the primary alkaloid comes from one of the Rhizoma coptidis. It has extremely significant antifungal action against Candida albicans, Candida tropicalis, and Candida glabrata. However, it was not proved that berberine possesses antifungal action in Candida albicans and Candida tropicalis.[27]

Indonesia contains a diverse range of plants that have the potential to be employed as alternative materials in the treatment of infectious illnesses. The Mangrove is a plant that is commonly distributed and has been shown to have several advantages. This plant may be found in a number of countries, including Vietnam, China, Cambodia, Malaysia, Singapore, Australia, and, of course, Indonesia. This plant has long been used as an anti-asthmatic, anti-diabetic, anti-rheumatic, and antibacterial remedy. Terpenoids, alkaloids, fatty acids, and flavonoids are among the chemical compounds found in mangroves. In addition, Mangrove leaves ethanol extract has antibacterial activitiy to periodontopathogen bacterias *in vitro*.[28] The flavonoid content of mangroves can impede spore formation, and flavonoids can combat pathogenic fungus in people and have been proven to decrease pathogen growth in opportunistic diseases such as Candida. Flavonoids have bacteriostatic qualities and, at greater concentrations, may kill both gram-negative and gram-positive bacteria.[29] Furthermore, this study aimed to investigate the anti-fungal ability of Mangrove Ethanol Extract (MEE) (*Aegiceras corniculatum*) to *C. albicans* isolated from Oral Candidiasis HIV/AIDS patient *in vitro.*

Materials and methods

This study protocol obtained the ethical clearance by Universitas Airlangga Surabaya, Faculty of Dental Medicine Health Research Ethical Clearance Commision with appointment number: 494/HRECC/FODM/VIII/2021.

Mangrove samples were collected at the MECoK Ecopark on the Diponegoro University Campus in Jepara. Fresh mangrove leaves with no signs of damage (physical or illness) were picked and preserved in dark plastic samples in a cold box to minimize metabolite degradation due to light and temperature. The leaves, stems, roots, and flowers were gathered to be used as identifying keys. The samples were then produced at Diponegoro University's Natural Product Laboratory. The leaves are cleaned by washing them in running water to eliminate any contaminants. The leaf sample was trimmed with scissors and weighed until it reached a weight of 138 g for extraction. A single solvent maceration approach with ethanol at a ratio of 1:2 (w/v) was used to extract metabolites from mangrove leaves, with many solvent changes owing to solvent saturation. Shaker agitation (100 r.p.m.) was used to macerate the samples for 24 hours at room temperature (25oC). Following maceration, the organic solvent was extracted and concentrated using a rotary evaporator at 35oC. The MEE (*A. corniculatum*) was then saved for later examination.

The design of this study was a post-test only control group using basic randomized sampling. The smallest sample size was calculated using Lameshow's minimum sample formula, and four replications were acquired for each group as a result of the computation. There were 5 groups, each with 4 samples: (1) control negative group (plain SDA), (2) control doxycycline group, (3) MEE (*A. corniculatum*) concentration 25%, (3) 50% concentration group, (4) 75% concentration group, and (5) 100% concentration group. To produce the examination concentration, 1 ml of 1 percent CMC solvent was added to a concentration of MEE (*A. corniculatum*).

C. albicans was detected from stadium HIV/AIDS patients who were assessed clinically but did not seek fungal infection therapy. A swab specimen was obtained from the 1/3-posterior of the tongue dorsum, and the swab stick was incubated for 24 hours at 37° C in Sabouraud's broth. The same patient's oral rinse specimen was obtained by forcing the subject to rinse for 15 seconds with 10 mL phosphate buffer saline (PBS). The oral rinse specimen was centrifuged, and the pellet was submerged in Sabouraud's broth for 24 hours at 37° C. The liquid medium

was poured over Sabouraud's dextrose agar and incubated for another 24 hours at 37° C after being stirred with a cotton swab stick. The agar medium were then visually and subjectively inspected to compare colony growth. The isolated Candida albicans was then stored for future research at the Dental Medicine Research Center, Faculty of Dental Medicine, Universitas Airlangga.[30]

Sabouraud dextrose agar (SDA) was dissolved in 20 ml of distilled water in Erlenmeyer then heated on a hot plate until boiling and a clear solution was obtained. Then it was poured into several test tubes, sterilized in an autoclave at 1210C for 15 minutes, then tilted at 300 and allowed to harden. Fungal colonies were taken from available pure cultures, carried out aseptically with a loop needle and streaked on the culture medium. For the manufacture of the Negative Control Solution, a 1% CMC solution was used. It was prepared by: 1 g of CMC was weighed and aquadest was added to 100 ml and then shaken until homogeneous.

Preparation of fungal suspension for anti-fungal ability was performed by culturing *C. albicans* in agar sloping media suspended with NaCl. Then, it is taken sufficiently and put into the seedling medium. Then mixed and adjusted for turbidity equal to Mc.Farland's solution. The antifungal activity test was carried out with SDA as poured into a petri dish and allowed to harden. On the surface of the base layer were placed 5 paper disks arranged in such a way that there was a good area to observe the inhibition zone that occurred.

SDA containing the tested *C. albicans* suspension was poured into a petri dish around the paper disk. Remove the paper disk from the petri dish so that a well is formed which will be used for the test solution, positive (+) control solution and negative (-) control solution. Drops of test solution for dry ethanol sample extract, ethanol wet sample extract, positive (+) control solution and negative (-) control solution, repeat it in quadriple in the same way then incubated in an incubator at 37^0C for 1x24 hours. Observation of the inhibition zone around the well and then measuring the diameter of the inhibition zone horizontally and vertically using a digital scale ruler. To find out whether there is an effect of the diameter of the inhibition zone on the growth of C. albicans, a statistical test was performed using the one way analysis of variance (ANOVA) test with the Statistical Package for social science 20.0 software (IBM corporation, Chicago, US).

Results

In this study, it was discovered that 100%, 75%, 50%, and 25% MEE (*A. corniculatum*) had no antifungal efficacy against *C. albicans* isolated from HIV/AIDS (Figure 1). Furthermore, Doxycyline, an antibacterial medication, exhibited no antifungal action against *C. albicans* isolated from HIV/AIDS patients. To limit the development of *C. albicans* isolated from HIV/AIDS, there was no significant difference in inhibitory zone between 100%, 75%, 50%, and 25% MEE (*A. corniculatum*), control negative group, and doxycycline group.

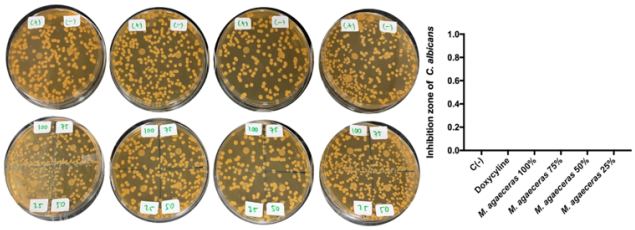

Figure 1. Mangrove Ethanol Extract (MEE) (*A. corniculatum*) did not have an inhibitory zone against *C. albicans* isolated from HIV/AIDS patients.

Discussion

Oral candidiasis is an infection induced by the over-cultivation of a fungus that ordinarily presents in tiny quantities. Candida albicans infection is often an opportunistic infection, characterized by the patient's immunocompromised state, in which the patient's immune system is impaired, causing the natural flora that should be commensal to become pathogenic.[30,31]

Each cell of the fungus Candida albicans has a cytoplasm that includes a plasma membrane, periplasm, cell wall, and certain extracellular structural components. The fungal protoplasm is protected by the cell wall from the external environment and growth stimuli, as well as from cell resistance, form, and different cell interactions. The plasma membrane components of fungal cells are composed of a phospholipid bilayer interspersed with globular proteins that regulate nutrient input and exit and act as selective barriers or translocation barriers. In

contrast to cholesterol in animal membranes and phytosterols in plants, ergosterol is the most abundant sterol discovered in fungal membranes.[32]

Periplasm is the gap between the plasma membrane and the cell wall. Fungal cell walls are made up of a variety of polysaccharides, including (chitin, glucan, mannoprotein, chitosan, chitin, cellulose). Because proteins and glycoproteins are smaller in size, they have a dynamic structure, and qualitative variations occur in various morphological forms of the same species and in response to environmental stress factors.[32] The antifungal mechanism can work by killing harmful fungus cells. Antifungal chemicals work by neutralizing enzymes involved in fungal invasion, disrupting fungal cell membranes, and inhibiting fungal enzyme systems, interfering with the creation of hyphae tips and affecting nucleic acid and protein synthesis.[25,27]

Saponin chemicals have the capacity to disturb the integrity of cell membranes in fungi, resulting in cell membrane destruction and the release of many essential components from within fungal cells such as proteins, nucleic acids, and nucleotides. Steroid chemicals have the ability to limit fungal growth either through the cytoplasm or by interfering with the formation and development of fungal spores. Tannin chemicals have the capacity to block the synthesis of chitin, which is employed in the building of cell walls in fungi, as well as damage cell membranes, so inhibiting fungal growth. Terpenoid chemicals can suppress fungal development by interfering with nutrition transfer, causing cell death.[33]

Mangroves contain the aforementioned bioactive compounds and have been shown to have antimicrobial. Mangroves also have metabolites that are useful in toxicology, pharmacology, and ecology, such as alkaloids, phenols, steroids, terpenoids, and flavonoids.[28] Several mangrove extracts were able to reduce the growth of Candida albicans, albeit with modest inhibition values when compared to positive controls using fluconazole. This might be owing to a poor solvent and extraction process, resulting in a lack of pure bioactive chemicals for application.[34]

The test of Mangrove inhibition of *Avicennia marina* against A*spergillus fumigatus, C. albicans,* and Mucor sp. was described by previous study. By removing the roots, fruits, and seeds By comparing extracts with different solvents such as ethanol, ethyl acetate, petroleum ether, chloroform, and water. The inhibition test with root extract diluted in ethanol and subsequently applied to the fungus Candida albicans revealed no inhibition with a lower MIC value (0.25 0.01 mg/mL) than the positive control fluconazole (0.6 0.03 mg/mL) as well as a number of other solvents.[35] Resistance of this fungus to the content of mangrove extract can arise owing to a number of mechanisms, including changes in the quality or quantity of target enzymes in medications and changes in plasma membranes in cells. Changes in the cellular content of the target enzymes caused by gene overexpression resulting in a rise in ergosterol produce poor azole penetration due to changes in membrane sterol or phospholipid composition and decreased permeability.[35,36] Other aspects, of course, can be induced by the extraction process, solvent type, extract concentration, and sample site, even if the plant contains secondary metabolites that should be able to suppress the fungus.[37,38]

Conclusion

According to the findings of this study, Mangrove Ethanol Extract (*A. corniculatum*) did not have an inhibitory zone against *C. albicans* isolated from HIV/AIDS patients *in vitro*. However, further study is still needed to investigate antifungal ability of Mangrove Ethanol Extract from other species to the others candida species.

Declaration of Interest

The authors declare there is no conflict interest in this study.

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