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Antifungal Activity Of Total Protein From Moringa Leaves (Moringa Oleifera) Against Candida Albicans

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Abstract

Moringa oleifera is a natural plant that grows abundantly in the natural laboratory of Universitas Islam Negeri Fatmawati Sukarno Bengkulu. Its leaves contain substantial amounts of protein and may have antifungal potential. Candida albicans is one of the fungi associated with skin infections, particularly in tropical countries such as Indonesia, where humidity is high. The use of synthetic antifungal drugs may cause unwanted effects and contribute to the development of resistance. Therefore, research into natural substances with antifungal activity is important. This study aimed to examine the antifungal activity of total protein extracted from M. oleifera leaves against C. albicans. The research procedures included protein extraction using salting-in and salting-out methods, fungal rejuvenation, and antifungal testing using the agar-well diffusion method. The test sample was water-soluble leaf protein at concentrations of 100, 300, 600, and 900 ppm. The results showed differences in inhibition-zone diameters among the protein concentrations. One-way ANOVA followed by Duncan's Multiple Range Test indicated that the 900 ppm concentration was the most effective treatment against C. albicans, producing an inhibition zone of 12.41 mm and falling into the strong antifungal-activity category.

Keywords Moringa Oleifera, Protein, Antifungal, Candida Albicans

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INTRODUCTION

Bengkulu is one of the provinces in Indonesia with rich biodiversity. Its tropical climate and fertile geographical conditions provide considerable opportunities for research into the potential of plants such as Moringa (Moringa oleifera). Moringa is a shrub that grows well in the natural laboratory of the Science Education Study Program at Universitas Islam Negeri Fatmawati Sukarno Bengkulu. Its leaves are small, approximately fingertip-sized, oval-shaped, and compound (Sari, 2017). The use of Moringa leaves has generally been limited to young leaves consumed as a vegetable. Leaves are, however, nutrient-dense plant parts. Moringa leaf flour has been reported to

contain a relatively high crude/total protein content of 28.7% (Teixeira, 2014).

A group of proteins interacts specifically with particular carbohydrates; these include water-soluble proteins and lectins (Lam & Ng, 2011). Carbohydrates are major components of cell structures in both prokaryotes and eukaryotes. Protein–carbohydrate binding may alter cell-membrane permeability. The cell membrane maintains the ionic composition of the cytoplasm, which is essential for cellular function. Changes in membrane permeability can disrupt nutrient transport and inhibit the activity and biosynthesis of specific enzymes required for metabolic reactions. As a result, cells may become deprived of nutrients needed for growth and may eventually undergo lysis (Haryanto & Muslim, 2014). These biological properties indicate that water-soluble proteins warrant further investigation as potential antifungal agents.

Fungi can cause harm to living organisms and materials. One fungus of concern to human health is Candida albicans, a yeast associated with infections, including skin infections. Such infections are common in tropical countries such as Indonesia, where humidity is high. Morace et al. (2014) reported that Candida species can develop high levels of resistance to azole antifungal agents, including fluconazole, itraconazole, posaconazole, and voriconazole. Resistance may lead to treatment failure and other problems. Alternative treatments, including naturally derived substances, are therefore worth investigating. Plant-based materials such as M. oleifera are readily available and may offer useful alternatives, although their safety and efficacy require scientific evaluation.

Charungchitrak et al. (2010) reported that water-soluble proteins can act as antifungal agents against Colletotrichum cassiicola, Exserohilum turcicum, and Fusarium oxysporum. In addition, Sari (2015) concluded that water-soluble protein from kabau seeds (Archidendron microcarpum) can inhibit microorganisms, including Gram-positive and Gram-negative bacteria. Research and development concerning antifungal agents have received relatively less attention than antibacterial research. This study was therefore conducted to determine the antifungal activity of water-soluble protein extracted from M. oleifera leaves against C. albicans.

METHOD

Extraction of water-soluble protein from Moringa oleifera leaves

Clean, selected Moringa leaves (50 g) were weighed, ground into a paste, and homogenized in 50 mL of Tris-HCl buffer (pH 10) using the salting-in method. The sample was kept under cold conditions for 24 hours. The homogenate was filtered, and the filtrate was centrifuged at 3,500 rpm for 15 minutes at 4°C. The pellet from the first centrifugation was discarded and the supernatant was collected. The supernatant was precipitated with 70% saturated ammonium sulfate at a 1:1 ratio using the salting-out method. A second centrifugation was then performed at 13,500 rpm for 30 minutes at 4°C.

The pellet obtained from the second centrifugation was used for the antifungal activity test (Sari, 2015).

Antifungal activity test

The testing procedure included sterilization of the agar medium, equipment, and materials; fungal rejuvenation; antifungal activity testing; and measurement of the inhibition-zone diameter. To prepare Potato Dextrose Agar (PDA), 5 g of PDA medium was placed in a 250 mL Pyrex Erlenmeyer flask and distilled water was added to a final volume of 125 mL. The equipment and materials were sterilized in an autoclave (HY 230S HUNG YI) at 127°C for 30 minutes, followed by UV exposure in a laminar airflow cabinet (NU 126 300E).

Before testing, the fungus was rejuvenated by adding 3 mL of sterile physiological saline (0.9% NaCl) to a test tube. A fungal suspension equivalent to 0.5 McFarland standard, or approximately 1.5×10^8 CFU/mL, was then prepared. The fungal suspension was spread over solidified PDA. Wells were made in the agar plates using a cork borer. The test samples consisted of water-soluble protein at four concentrations (100, 300, 600, and 900 ppm), distilled water as the negative control, and ketoconazole at 300 ppm as the positive control. A 10-microliter volume of each sample was added to each well using an Eppendorf micropipette. The plates were incubated at 37°C for 24 hours. Inhibition-zone diameters were measured using a vernier caliper, with three measurements taken from different viewing angles (Sari, 2015).

FINDINGS AND DISCUSSION

Extraction of water-soluble protein from Moringa oleifera leaves

The extraction process was intended to obtain protein from Moringa oleifera leaf material using a water-based solvent. It involved four main stages: sample preparation, preparation of the extraction solution, protein extraction, and removal of non-protein components. Before extraction, the leaves were ground and homogenized to disrupt cellular structures and release proteins. The prepared sample was mixed with the extraction solution and incubated under specified temperature and time conditions to facilitate protein release from the sample matrix. The use of water as the solvent enabled soluble proteins to enter the extraction solution.

The extraction solution was Tris-HCl buffer at pH 10. The buffer helps maintain pH during cell lysis, limits the activity of protein-degrading enzymes, and reduces changes in protein molecules. Extreme pH conditions may cause denaturation and proteolysis (Sari, 2019). The first centrifugation, conducted at 3,500 rpm for 15 minutes, separated impurities from the sample solution. The extract obtained after maceration remained cloudy and contained cell debris, organelles, and cellular macromolecules, including proteins, which could then be separated by centrifugation (Baynes & Dominiczak, 2014).

Further processing was used to separate proteins from non-protein components such as lipids, nucleic acids, and carbohydrates. Precipitation was carried out using 70%

saturated ammonium sulfate. Salting-out separates proteins according to their solubility at high salt concentrations (Duong-Ly & Gabelli, 2014). At high salt concentrations, ionic strength increases and salt ions bind more water molecules. Consequently, fewer water molecules remain available to interact with proteins, increasing protein–protein interactions and causing precipitation. Different proteins have different solubilities in saturated ammonium sulfate. Globulins may precipitate as a pellet, whereas albumins may remain dissolved in the supernatant. The second centrifugation was performed at a higher speed and for a longer period—13,500 rpm for 30 minutes—to separate precipitated protein from the solution. Charungchitrak et al. (2011) used a higher ammonium sulfate concentration (90%) when studying lectin from Archidendron jiringa seeds as an antifungal agent against E. turcicum, F. oxysporum, and C. cassiicola.

All extraction stages were maintained under cold conditions at 4°C. This was based partly on Sudjadi et al. (2004), who reported that tested protein was more stable at 4°C than at higher temperatures. The method was therefore intended to preserve the quality of M. oleifera leaf protein. The final protein extraction yield in this study was 12.44%. This was higher than the yields reported by Yaméogo et al. (2011), who obtained 11.9 g of protein per 100 g of fresh leaves, and Abbas et al. (2018), who reported 8.1 g/100 g of material, but lower than the 30.3% reported by Moyo et al. (2011). The difference may be related to sample preparation: Moyo et al. used dried leaves, whereas the present study used fresh leaves. The extracted water-soluble protein was subsequently tested for antifungal activity.

Figure 1. Centrifugation results: (a) first stage and (b) second stage.

Antifungal activity test

The results showed inhibition zones around the wells containing Moringa oleifera leaf protein at each tested concentration. In contrast, distilled water, which served as the negative control, did not produce an inhibition zone. This indicates that the solvent itself did not affect antifungal activity and that the observed activity originated from the test solution rather than the solvent. Distilled water was used as the negative control and in preparing the protein concentrations and the leaf sample solution during the first centrifugation stage. Ketoconazole, an azole antifungal agent at 300 ppm, was used as the positive control. It is used against Candida infections affecting the skin, hair, nails, and other body areas.

The mean inhibition-zone measurements indicated that higher concentrations of water-soluble M. oleifera leaf protein produced larger inhibition zones, meaning that a wider area was free from fungal growth. This finding is consistent with Pelczar and Chan (1988), who stated that increasing the concentration of an antimicrobial substance generally increases its inhibitory activity. The inhibition-zone measurements were analyzed using one-way ANOVA. Because the treatments differed significantly, Duncan's

Multiple Range Test (DMRT) was subsequently applied. The results are presented in Table 1.

Table 1. Inhibition-zone diameter against C. albicans

No.	Treatment	Replicates	Mean (mm)	SD	DMRT group / activity
1	Water-soluble protein 100 ppm	3	10.30	0.15	a / Strong
2	Water-soluble protein 300 ppm	3	10.66	0.26	a / Strong
3	Water-soluble protein 600 ppm	3	10.88	0.16	a / Strong
4	Water-soluble protein 900 ppm	3	12.41	0.47	b / Strong
5	Ketoconazole 300 ppm	3	24.45	0.39	c / Very strong

The findings demonstrate that Moringa oleifera leaf protein has antifungal activity against Candida albicans. Fungal growth was inhibited after treatment with the different concentrations of water-soluble M. oleifera leaf protein. This suggests that the protein has strong antifungal activity against C. albicans. The finding is relevant to the development of potential antifungal materials because C. albicans is associated with fungal infections in humans.

The Duncan test showed that the effects of the 100, 300, and 600 ppm water-soluble protein concentrations were not significantly different from one another. The 900 ppm concentration differed significantly from ketoconazole at 300 ppm and from the three lower protein concentrations. All water-soluble protein concentrations fell into the strong antifungal-activity category, whereas ketoconazole was categorized as very strong. An antifungal agent is considered highly active when a relatively low concentration produces a large inhibition zone (Ningsih et al., 2017). Thus, among the protein concentrations tested, 900 ppm was the most effective against C. albicans.

Moringa oleifera leaf proteins may inhibit fungal cells through several mechanisms. Djunaedy (2008) explained that antifungal compounds may damage fungal cell membranes, neutralize enzymes involved in fungal invasion, inhibit fungal enzyme

systems and disrupt hyphal-tip formation, and affect nucleic-acid and protein synthesis. Water-soluble proteins reported in M. oleifera leaves include chitinase, Moringa oleifera lectin (MOL), moringin, defensin-like proteins, and protease inhibitors. These proteins may act through disruption of cell structures, enzymatic inhibition, immune-response stimulation, and damage to cellular components. The present experiment, however, did not isolate or individually characterize these proteins; the mechanisms discussed here provide a possible explanation rather than a mechanism demonstrated directly by this study.

Chitinase is an enzyme capable of breaking down chitin, a specific polysaccharide composed of N-acetylglucosamine (GlcNAc) units linked by β-(1,4)-glycosidic bonds. Chitin is an important component of fungal cell walls, including those of Candida albicans. The fungal cell wall protects cells against osmotic pressure, mechanical damage, and pathogens. Chitin contributes mechanical strength and forms a complex matrix with glucans and proteins. It also plays a role in fungal growth and development, hyphal formation, septum formation, and interactions with the surrounding environment. Chitinase breaks β-(1,4)-glycosidic bonds in chitin. Damage to the cell wall may compromise its ability to withstand osmotic pressure, alter cell integrity and permeability, cause leakage of essential substances, disrupt nutrient transport, and ultimately inhibit fungal growth and replication. Chitinase activity may also generate chitin-degradation products that act as molecular signals and stimulate immune cells, such as macrophages and dendritic cells, to recognize and eliminate fungal pathogens (Cruz-Martín et al., 2023).

Research on chitinase has been conducted in many laboratories over the past three decades, largely because of the antifungal properties of chitinases (Yan & Fong, 2015). The microbial production of chitinase has also attracted worldwide attention (Veliz et al., 2017).

Lectin is another water-soluble protein component associated with Moringa oleifera. Moringa oleifera lectin (MOL) has a tertiary structure similar to legumin, a storage protein commonly found in plants. MOL consists of two identical subunits linked by disulfide bonds. Each subunit contains domains forming β-sheet folds and α-helices. MOL has an affinity for certain sugars and carbohydrates. Its specific sugar-binding sites enable strong interactions with carbohydrate groups. Lectins can bind to specific carbohydrate groups on fungal cell membranes, including glycoproteins. These glycoproteins are located on the fungal cell surface and contain mannan polysaccharide chains attached to a protein core; they contribute to recognition and interaction with the environment. Such binding may alter membrane permeability and disrupt membrane structure and function. The cell membrane protects cellular contents and regulates the movement of molecules required for cell survival (Alfiah et al., 2015). Changes in membrane permeability may interfere with specific enzymes, metabolism, and nutrient absorption, causing nutrient deficiency

and eventually cell lysis.

Figure 2. Effect of water-soluble M. oleifera protein on the inhibition-zone diameter of C. albicans.

The main difference between total protein from Moringa leaves and antifungal medicines concerns their sources. Total Moringa leaf protein is plant-derived, whereas medicines are generally synthetic or semi-synthetic products. Moringa leaves also contain various nutrients. Ketoconazole, used as the positive control, is a commercial synthetic drug and showed strong antifungal activity against the fungus tested.

Ketoconazole belongs to the azole class and is used to treat fungal infections of the skin, hair, and nails. It is active against various fungi, including dermatophytes such as Trichophyton and Microsporum and yeasts such as Candida. Ketoconazole inhibits ergosterol synthesis by inhibiting cytochrome P450 enzymes involved in the ergosterol biosynthesis pathway. Ergosterol is an essential component of the fungal cell membrane. Disruption of ergosterol synthesis changes membrane structure, weakens membrane integrity, and interferes with normal fungal-cell function. Increased membrane permeability may allow essential substances to leak out and harmful substances to enter. This disruption of membrane function and the transport of essential compounds can cause metabolic imbalance, inhibit fungal growth, or lead to cell death (Hidayat et al., 2022).

Ketoconazole has been used for various fungal infections of the skin, hair, and nails, including tinea corporis, tinea cruris, tinea pedis, cutaneous candidiasis, pityriasis versicolor, and fungal nail infections. It is available in several dosage forms, including creams, lotions, shampoos, and tablets. Topical preparations are generally used for skin and hair infections, while oral tablets have been used for more extensive or difficult-to-reach infections. Potential adverse effects include skin irritation, redness, itching, or burning at the application site. Rare systemic effects may include liver dysfunction, hormonal disturbances, or allergic reactions. Medical conditions and other medicines may affect its use; for example, it may not be suitable for people with liver disease, and drug interactions may occur.

Much antimicrobial research has focused initially on antibacterial activity because bacterial and fungal cell walls have different structures. Bacterial cell walls contain peptidoglycan, whereas fungal cell walls contain chitin. These structural differences influence how test compounds interact with microorganisms. The structural and biological differences between fungi and bacteria make antifungal testing more complex and require a different approach. Previous research has extensively examined secondary metabolites as antifungal agents and has produced important findings. Research on proteins as antifungal agents may still be at an earlier stage and requires further work to identify and validate proteins with antifungal potential. Protein-based research can be more complex and resource-intensive than research on secondary metabolites because protein isolation, production, and characterization may involve complicated and costly

procedures, including recombinant DNA technology, protein expression, and purification. Protein targets may also have complex and specific modes of action, requiring detailed investigation of their functions in fungal biochemical pathways (Giner-Llorca et al., 2023).

Although many studies have examined secondary metabolites as antifungal agents, research on proteins remains important. Proteins are primary metabolites found in plant cells and play roles in numerous biological processes. In contrast, secondary metabolites may vary according to plant species, growing conditions, season, and environment. Research on total protein as an antifungal agent can provide a basis for addressing fungal infections and exploring applications in food preservation and other industries concerned with preventing fungal growth. Research on primary proteins and secondary metabolites can complement one another and contribute to the development of improved antifungal agents and future therapies.

CONCLUSION

The results show that Moringa oleifera leaf protein has antifungal activity against Candida albicans. The agar-well diffusion test showed significant differences in inhibition-zone diameters among the treatments. Duncan's test indicated that the effects of water-soluble protein at 100 ppm (10.30 mm), 300 ppm (10.66 mm), and 600 ppm (10.88 mm) were not significantly different. In contrast, the 900 ppm concentration (12.41 mm) differed significantly from ketoconazole at 300 ppm and from the three lower protein concentrations. All tested water-soluble protein concentrations were categorized as having strong antifungal activity, while ketoconazole showed very strong activity. Therefore, 900 ppm was the most effective concentration of water-soluble M. oleifera leaf protein against C. albicans among the concentrations tested. These findings indicate the potential of M. oleifera leaf protein as a source for further antifungal research.

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