Role of Ziziphus mauritiana Lam. on Oral Candidiasis and its Relation to the Antibody Response, Blood Electrolyte, and Liver Profile

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Abstract

Candida albicans is the primary agent in the pathogenesis of oral candidiasis involving liver metabolism, immune cells, and blood electrolytes. Ziziphus mauritiana Lam. was reported as an antifungal and an immunostimulator in the regulation of blood physiology.

The aim of this study is to evaluate the effect of Z. mauritiana Lam. on oral mucosal healing after infection with C. albicans, which further affects the immune response, blood electrolytes, and liver defense system. The mucosa, immune cells, and liver samples of 20 mice (Rattus novergicus) were examined via H&E staining. The blood electrolytes were investigated using the Caretium X931FT electrolyte analyzer. Z. mauritiana Lam. was extracted with ethanol, and its compounds were analyzed with GCMS. Meanwhile, the bioactivity was analyzed using the Molinspiration Cheminformatics software. Z. mauritiana Lam. can repair and prevent damage to epithelial cells, fibroblasts, and keratinization in the mucosa, as characterized by the increased blood immune cell response, blood electrolyte stability, and liver cell function.

The experimental period was 7 and 14 days. At the same time, potassium increased in contrast to the average value. Z. mauritiana Lam. presented high biological activity on all compounds (>.00), especially GPCR ligands, nuclear receptor ligands, protease inhibitors, and enzyme inhibitors. This ligand inhibits pathogen interaction with host cells, subsequently helping with the healing process and damage of C. albicans cells. Z. mauritiana Lam. may improve the healing of oral mucosal infections, reduce the frequency of C. albicans, and maintain immune cell response, liver metabolism, and blood electrolyte balance as mediated by the biological activity.

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Introduction

Fungal infection incidences are rising in the human population; this phenomenon may contribute to the morbidity and mortality of healthy individuals and worsen the conditions of immunocompromised individuals[1]. Estimates suggest that 70% to 80% of patients are infected with candidiasis due to *Candida albicans*[2]. Oral mucosa is the most common site of candida infection. The effects of *C. albicans* infection affect the body's defense system because oral candidiasis infection involves the metabolic response of organs for strengthening the host immune system[3]. Host defense against *C. albicans* relies heavily on innate and adaptive immune responses to neutralize fungal infections. Th1 and Th17 cellular responses offer immune defense against *C. albicans* infection. In addition, the role of these antibodies is to maintain tissue homeostasis [4].

Oral candidiasis is an opportunistic infection with symptoms that include thrush and creamy white lesions on the tongue, mucosa, palate, gums, and throat. Moreover, candidemia can increase fever incidences, which is challenging for antifungal treatments[3].

Candidemia causes septic shock, including low blood pressure, fast heart rate, and heavy breathing. This phenomenon is called invasive candidiasis because it involves serious

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infections that affect blood circulation, heart activity, and brain, eye, bones, and liver function[5]. The liver is one of the metabolic organs that most frequently changes after exposure to *C. albicans* fungal infection. In addition, the liver plays a role in the immune response and neutralizes the metabolic wastes of the antigen–antibody reaction during the elimination process. The impact of candida yeast infection on the liver causes abscess formation and liver sinusoid damage, and it interferes with the activity of the immune system against fungal invasions [6]. Moreover, candida yeast infections affect heart pressure and blood pressure and increase the body temperature, subsequently affecting the blood electrolytes [7].

The increasing activity of IgG, IgA, and IgM are a good indicator of increased candidemia infection [8]. This infection threatens the immune system and causes changes in the oral mucosa, blood circulation, and liver [9]. Clinical changes in the oral mucosa due to candidemia infection are characterized by pain and difficulty of swallowing. Meanwhile, changes in the blood flow system are characterized by an increase in sodium and potassium and a weakened response of blood immune cells [10]. The changes in the liver system are characterized by the increased infiltration of inflammatory cells and hyperemia and the occurrence of fat degeneration [11]. For a number of these effects, they can threaten the host if they last for a long time.

The use of antifungal drugs, such as ketoconazole, fluconazole, voriconazole, and terbinafine, cause a certain level of hepatotoxicity or clinically apparent liver injury with jaundice [12]. In addition, the treatment of candida infection causes toxicity to host cells. Researchers have continued to study comprehensive antifungals for avoiding secondary infections or the effects of toxicity on host cells and preventing excessive immune responses. The use of natural ingredients has been a continuous commitment in pharmacology as a means of finding antifungal drugs that do not cause side effects on the mucosa and metabolic organs.

Ziziphus mauritiana Lam. reportedly has good pharmacodynamic and pharmacokinetic properties. This plant is often used in the treatment of inflammation and can act as an antibacterial and antifungal [13,14].

As for the state-of-the-art elements of this research, the previous studies have not investigated the relationship of *C. albicans* infection to the oral mucosa associated with changes in blood electrolytes and the related impact on the metabolic organs (e.g., liver) in relation to the use of *Z. mauritiana* Lam. Moreover, the frequency of *C. albicans* and the histological repair of the oral mucosa have not been widely reported. This study attempts to evaluate the relationship between decreased infection and development of *C. albicans* with respect to oral mucosal healing, antibody response to blood electrolyte activity, and liver metabolism after the administration of *Z. mauritiana* Lam. ethanol extract to *Rattus norvegicus* infected with *C. albicans*. The bioactivity data from using *Z. mauritiana* Lam. can serve as a reference for the healing process of fungal infection on the oral mucosa.

Materials and methods

2.1 Animal Model Trial

This study involved only a post-test control group combined with a descriptive–analytic approach. Twenty rats (*R. norvegicus*) aged 8–10 weeks and 150-200 g of bodyweight were used as the samples. Before the treatment, the rats were acclimatized for 7 days in a conducive atmosphere. The process of euthanasia in mice by using intraperitoneal ketamine LD50 was adopted [15]. The animal carcasses were burned in an incinerator (Industrial Estate, Canning Rd., Southport PR9 7SN, United Kingdom) at 850°C.

2.2 Plant Material

The *Z. mauritiana* Lam. samples were obtained from Aceh Farm School, Aceh Besar District, Province of Aceh, Indonesia (5.570704102637194, 95.35980633540842). *Z. mauritiana* Lam. was extracted at the Chemical Laboratory, Education Faculty, Universitas Syiah Kuala, Darussalam Banda Aceh Indonesia (voucher no. D1101). The assay materials were collected by the author from the Oral Biology Laboratory, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Indonesia.

2.3 Extraction and Gas Chromathography–Mass Spectrometry (GCMS) Assay

Z. mauritiana Lam. leaves (total of 1 kg) were chopped and macerated in 5 L of 96% ethanol for 24 hours and stirred every 4 hours and then decanted and filtered. The residue was

further macerated using 96% ethanol for 48 hours. Then, the filtrate was evaporated with a rotary vacuum evaporator until a concentrated extract was obtained. The extract was heated at 45 °C to remove the remaining ethanol.

The chemical compound of *Z. mauritiana* Lam. was identified via GCMS. The GCMS apparatus (Shimadzu QP2010PLUS, Tokyo, Japan) was run between 80 °C and 200 °C. Then, the obtained compounds were stored for 1 minute at 80 °C. The flow rate was set at 5 °C/minute and 200 °C for 20 minutes. The FID temperature was 300 °C, and the injection temperature was 220 °C. The nitrogen carrier gas was estimated at 1 mL/min flow rate, 1:75 split ratio, and pressure of 116.9 kPa. The column length was set as 30 mm, the diameter was 0.25 mm, and the flow rate was 50 mL/min.

The treatment for chemical stability determination was started by injecting the ethanol extract of *Z. mauritiana* Lam. into an injector, subsequently allowing it to evaporate, for gas-to-column separation. Then, the ionization process was carried out. A detector was used to capture the resultant ionic fragments for producing the mass spectra. The research data were processed and then compared with the chemical values of the standard reference material (SRM) database. Similarity percentages were expressed as the analyte values for comparison with the standard library spectra, and both data were declared identical if the system could capture ratios of >90%.

2.4 C. albicans Culture and Staining

A mucosal swab resuspended in a buffer solution was taken using an oasis needle under sterile conditions and smeared on a selective medium (CHROMagar, Paris, France). A one-liter composition of the medium consists of chromopepton (10.0 g), glucose (20 g), chromogen mix (2 g), chloramphenicol (0.5 g), and agar (15.0 g), with pH of 6.0+/−0.3. Then, *C. albicans* was cultured at 37 °C for 48 hours. *C. albicans* growth was specifically identified by observing the presence of bright green colonies [16]. The number of growing colonies on triplicate plates was counted [17].

The *C. albicans* cells were observed using the gram staining method following the work of Gani, 2017 [18]. Aseptically, one needle loop of the *C. albicans* culture was taken from the surface of the slide. Afterwards, the sample was flattened with an ose needle by using sterile distilled water. The sample was fixed by heating the glass slide over a Bunsen flame three times. This approach of fixation could kill the *C. albicans* while retaining the cell's shape and components. Crystal violet dye was dropped evenly on the fungus for 20 seconds. Then, the sample was washed with sterile distilled water before drying. Lugol's iodine solution was dropped onto the fungus surface for 60 seconds, rinsed, and dried. Then. the sample was dripped with decolorizer solution (alcohol acid), washed with distilled water, and dried. The safranin dye was evenly distributed over the bacteria for 20 seconds and allowed to dry in the last step. The surface was affixed with a cover glass prior to observation under a microscope.

2.5 Histopathology Assay

The histopathological evaluation of the mucosa and liver was carried out according to the method of Gani, 2015 [19]. The mucosa and liver of the euthanized rats were taken for histopathological preparation. The mucosa with a thickness of approximately 5 mm was excised to the submucosa, while the liver was separated from the other organs. Both organs were fixed with a 10% neutral-buffered formalin solution. An organ trimming was performed, and the obtained specimens were placed on a tissue cassette. For the next stage, the dehydration process was implemented by applying two rounds of acetone for 1.5 hours, followed by purification by using two rounds of benzol for 1.5 hours. The printing process or paraffinization was carried out with benzol + paraffin (1:1) for 1.5 hours and paraffin for 1.5 hours. The preparation was inserted into a printing device/block containing a half volume of paraffin, and the prepared sample was affixed to vertical and horizontal collars, allowing the cross section to be attached to the paraffin base. After the sample was solidified, paraffin was added again until the block/mold was full. The sample was left for paraffin hardening.

The paraffin blocks were cut into 5 mm thin strips by using a microtome. The resultant ribbon-like strips were spread over warm water at 46 °C and immediately lifted to stretch the piece and avoid folding while eliminating the creases caused by the cutting. The sample was then removed and placed on an object glass and dried for 18 hours in an incubator at 60 °C for the preparation of Mayer hematoxylin and eosin (H&E) stains. Forty-two rounds of Mayer hematoxylin staining was carried out for 8

minutes and then washed in running water for 30 seconds. The eosin staining was performed by immersing the sample in an eosin solution for 2–3 minutes and then washed with running water for 30 seconds. The samples were immersed in 95% alcohol and absolute alcohol (I and II) for ten times. Then, each sample was gradually immersed in absolute alcohol and xylol I for 1 minute, followed by xylol II for 2 minutes. Finally, the prepared tissue samples were enclosed in an object glass. Finally, the glass was dripped with Entellan adhesive, and histopathological observations were carried out using an electric microscope (Olympus, Tokyo, Japan).

2.6 Blood Smear Preparation and Staining

The blood immune cells and antibody profiles were examined via a thin blood smear approach based on the working principle adopted by Adewoyin (2014) [20]. First, a glass object with drops of blood was placed on a table or a flat surface, and the object–glass was affixed by pressing the left index finger on the edge of the glass without an identity sticker. Then, another glass object was prepared to serve as a booster. Then, a pusher glass object was placed on top of the second drop of blood at an angle of 45 degrees to enable the blood to spread to all ends of the pusher glass object. The object–glass pusher was pulled back by approximately 5 mm and then pushed forward while maintaining an angle of 45 degrees. The pushing glass object was never separated from the blood drop glass object. Then, the smear was dried at room temperature after soaking in 76% methanol for 30 minutes followed by 3% Giemsa solution for another 30 minutes. The sample was washed with running water and placed on an inclined position and allowed to dry. Further observations were performed under a microscope with 400× magnification.

2.7 Blood Electrolyte Assay

For the electrolyte examination of the serum, the ion-selective electrode method was adopted. The serum from approximately 5 mL of blood was used as a sample after being centrifuged for 3 minutes at a speed of 4000 rpm using an electrolyte analyzer (Happycare, Guangdong, China). The blood serum from the experimental animals was collected using a 0.5 mL syringe. The suction needle was inserted into the serum cup and its position was maintained until it could suck serum in the cup for ±2 seconds. The power button was pressed until the suction needle could be re-entered into the tool, and then the electrolyte levels in the serum were analyzed for ±30 seconds. Finally, the serum electrolyte levels appearing on the monitor screen were recorded as the results.

2.8 DPPH Antioxidant Assay

The antioxidant quality of *Z. mauritiana* Lam. was examined following the approach of Yusuf (2021) who used 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) [21]. The DPPH stock (125 µM) was obtained by combining 2.5 mg of DPPH dissolved in 50 mL of ethanol p.a. The sample (test material) and vitamin C were prepared as the standard, and 10 mg of each part were dissolved into 1 mL of DMSO. These two test materials were individually placed into 100 µL of 96-well plates, and 100 µL of DPPH was added. Meanwhile, 100 µL of ethanol p.a. was added to the negative control, and the sample was incubated at room temperature in the dark for 30 minutes. The results of the antioxidant activity were determined via spectrophotometry at a wavelength of 517 nm.

2.9 Bioactivity Score Analysis

The bioactivity and molecular properties were calculated on Molinspiration, a property engine software. The compound structure of *Z. mauritiana* Lam. extract was obtained using the ChemDraw Professional v.16© Cambridge soft software, and the 2D structure was determined. Meanwhile, the bioactivity and molecular score of *Chromolaena odorata* were calculated using Molinspiration v. 2018.10 [22].

2.10 Statistical Analysis

The comparative data obtained for the research variables were clarified via statistical analysis. The differences in concentrations for depicting the antioxidant quality of *Z. mauritiana* Lam. were analyzed via one-way ANOVA. Meanwhile, the blood cells and blood electrolytes were analyzed according to the Kruskal–Wallis test. The number of *C. albicans* colonies from both groups was analyzed by t-test. Pearson correlation was adopted to determine the relationship of *C. albicans* colony growth and the influence of the test material. Spearman's rho correlation was used to test the time relationship of the *C. albicans* growth colonies.

Results

Figure 1 shows the treatment group on

the day 7 (Figure 1A) with a thickened keratin layer. The epithelial cells in the buccal mucosa relatively neat and orderly, with less inflammatory cell infiltration. On day 14 (Figure 1B), the keratin layer was thinned, and the epithelial cells appear normal. Meanwhile, in the azole group, the keratin appears eroded on day 7 (Fig. 1C), and the inflammatory cells are decreased on day 14 (Fig. 1D). The epithelial cells are generally regular, and the keratin layer begins to thicken. Furthermore, the connective tissue is relatively dense.

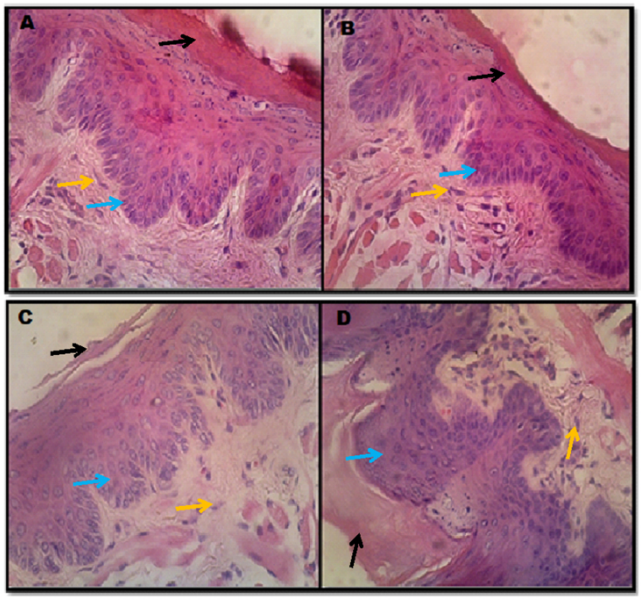


Figure 1. Histopathological profile of mucosa. Treatment group with Z. mauritiana Lam. at (A). 7 days and (B) 14 days. Treatment group with azole at (C) 7 days and (D) 14 days. Black arrows: keratin; blue arrows: epithelial cells; yellow arrows: fibroblasts. Magnification: 400×.

Group	Colony-forming unit (CFU)	
	Day 7	Day 14
Control	250	316
Treatment Group (***Z. mauritiana*** Lam.)	70	22
Treatment Group (azole)	67	32

Table 1. *C. albicans* isolated from oral mucosal infection.

Figure 2 shows the oral mucosa exposed to *C. albicans* at day 7. The infection is characterized by the presence of *C. albicans* cells on the oral mucosa. Colony calculations were carried out to determine the number of *C. albicans* for each treatment group (Table 1). The treatment group (*Z. mauritiana* Lam.) and the azole group were similar in preventing the spread of *C. albicans* on the oral mucosa at days 7 and 14. However, as observed from the image and the number of *C. albicans* colonies, the treatment group with *Z. mauritiana* Lam. had a better effect than the azole group (antifungal drug).

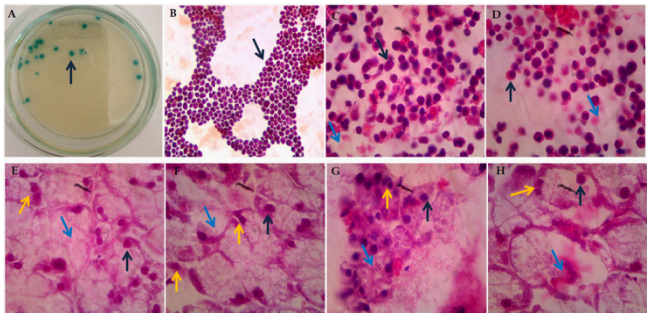
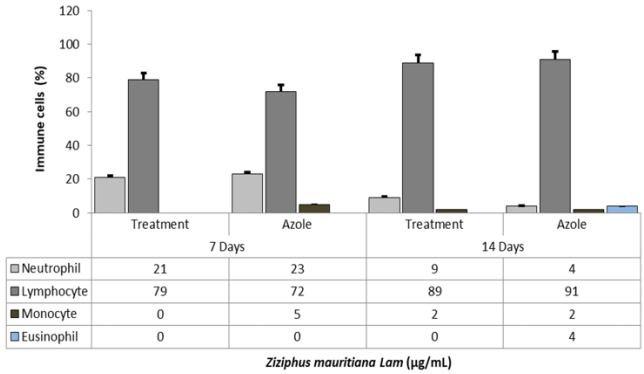


Figure 2. *C. albicans* on the oral mucosa of *R. norvegicus*. (A) *C. albicans* on CHROMagar selective medium. (B). *C. albicans* cells on gram stain. (C) *C. albicans* on the oral mucosa of the negative control group at day 7. (D) *C. albicans* on the oral mucosa of the negative control group at day 14. (E) *C. albicans* on the oral mucosa of the *Z. mauritiana* Lam. treatment group at day 7. (F) *C. albicans* on the oral mucosa of the *Z. mauritiana* Lam. treatment group at day 14. (G) *C. albicans* on the oral mucosa of the azole treatment group at day 7. (H) *C. albicans* on the oral mucosa of the azole treatment group at day 14. Black arrow: *C. albicans* colony or cell; blue arrow: destroyed epithelial cells; yellow arrow: damaged *C. albicans* cells. Magnification: 400×.

The t-test showed that the number of *C. albicans* colonies in the oral mucosa of *R. norvegicus* was significantly different ($p < .05$; .003). In particular, the follow-up independent sample t-test showed that the control group with the treatment (*Z. mauritiana* Lam.) showed significant differences ($p < .05$; .035). The differences were also significant between the control group and the azole group ($p < .05$; .046). Meanwhile, between the treatment groups with azole test material, the difference was non-significant ($p > .05$; .918). The Kruskal–Wallis test also determined that the differences in quantity of *C. albicans* colonies between days 7 days and 14 were non-significant ($p > .05$; .513).

The Pearson correlation test showed a negative relationship between the treatment groups in terms of the number of *C. albicans*

colonies (r = −.837). This finding means that the population of *C. albicans* in the oral mucosa of *R. norvegicus* was not affected by the type of treatment, but the presence of *C. albicans* was significantly different between the treatment groups (p=.037). Similarly, the Spearman's rho correlation test showed a negative relationship between treatment time and *C. albicans* population (r = −.293). This finding indicates that time does not affect the amount of *C. albicans* in the oral mucosa of the subject animals, as difference was non-significant between days 7 and 14 (p = .573).



	7 Days		14 Days	
	Treatment	Azole	Treatment	Azole
Neutrophil	21	23	9	4
Lymphocyte	79	72	89	91
Monocyte	0	5	2	2
Eusinophil	0	0	0	4

Figure 3. Blood immune cells from mucosal infection. On day 7 of the azole group, monocyte cells are found, and the number decreases at day 14. The same trend is found in the treatment group. The response of lymphocyte cells is enhanced on day 14 of both groups. Bar: immune cells; bar error: errors with percentages.

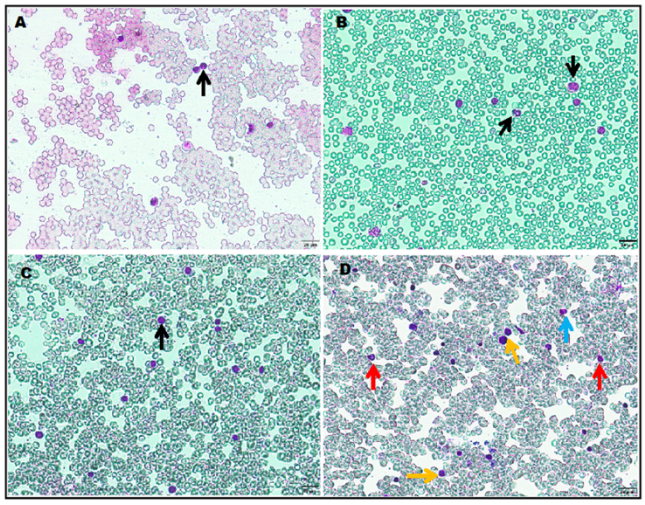


Figure 4. Histology of blood inflammatory cells. Treatment group with *Z. mauritiana* Lam. at (A) day 7 and (B) day 14. Treatment group with azole at (C) day 7 and (D) day 14. Black arrows: neutrophils; yellow arrows; lymphocytes; blue arrows: monocytes; red arrows: eosinophil. Calculation (%): number of immune cells based on antibody counters in multiples of 100%. Magnification: 400×.

Figure 3 shows the response of the immune cells to *C. albicans* during the infection phases of the azole and treatment groups. In general, the increase of lymphocytes is more apparent compared with those of the other immune cells. Figure 4 illustrates the presence of these immune cells in varying quantities. The response of the immune cells to the *C. albicans* antigen is shown in Figure 5. Antibodies are found in the blood serum, indicating the presence of the phagocytic process of antigen by the antibody.

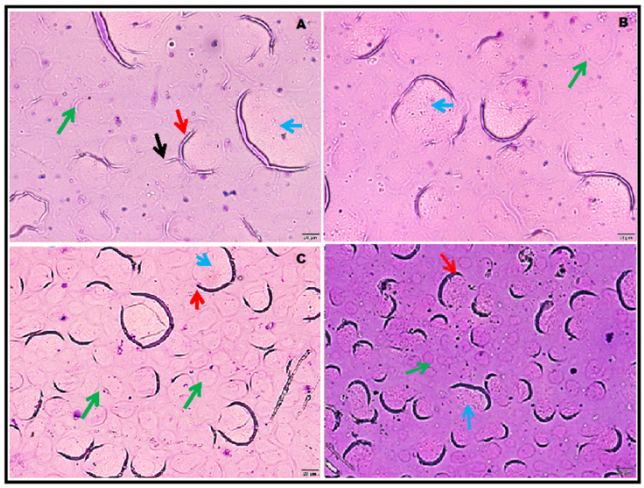


Figure 5. Blood serum antibody profile. Control group at (A) day 7 and (B) day 14. Treatment group at (C) day 7 and (D) day 14. Red arrow: fragment antigen binding; blue arrow: opsonized antigen; green arrow: remaining antibody. Some antibodies in the control and treatment groups underwent antigen phagocytosis. Magnification: 400×.

Blood serum electrolyte	Day 7 (mmol/L)		Day 14 (mmol/L)		Normal value (mmol/L)
	Azole (%)	Treatment (%)	Azole (%)	Treatment(%)	
Sodium	143 (17)	140,5 (17)	138 (16)	144 (17)	135-145
Potassium	6,7 (17)	6,85 (18)	7 (18)	5,55 (14)	3.5-5.5
Chloride	98 (17)	97,5 (16)	95 (16)	101,5 (17)	94-105

Table 2. Value of rat blood serum electrolytes after treatment with *Z. mauritiana* Lam.

The Kruskal–Wallis test results were used to determine the presence of immune cells by type between days 7 and 14, and the difference

was significant (p <.05:.006). However, no significant difference was observed between the azole and treatment groups between days 7 and 14 (p >.05:.596). Thus, the type of immune cell can be used as a reference for the occurrence of an immune response to the development of mucosal infections in experimental animal hosts.

Table 2 shows the varying blood electrolyte values between the control group and the treatment groups. The differences in values indicate an immune response against *C. albicans*. The values of sodium and chloride in the blood remained within normal limits in the control group and the treatment group at days 7 and 14. Meanwhile, the potassium increased in the control group and decreased in the treatment group at day 14. The t-test results showed that the differences in blood electrolytes in the treatment groups at days 7 and 14 days were non-significant (p >.314). The Kruskal–Wallis test analysis showed that the value of each type of blood electrolyte (sodium, potassium, and chloride) had no significant difference (p >.05;.538).

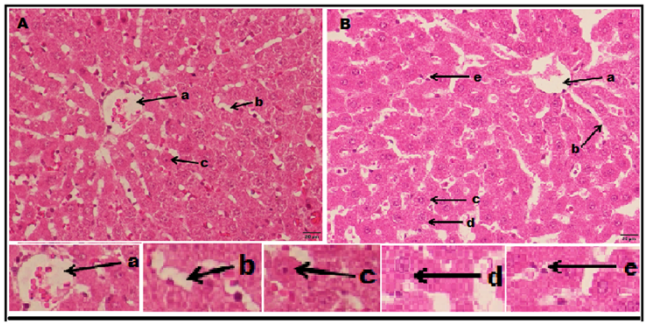


Figure 6. Histopathological profile of the liver in the treatment group at (A) day 7 and (B) day 14. (a) Vena centralis, (b) sinusoids, (c) hepatosit, (d) nucleus, and (e) Kuffer cells. Magnification: 400×.

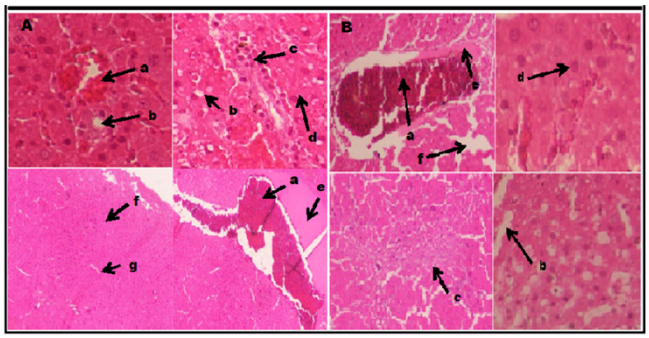


Figure 7. Histopathological profile of the liver in the control group at (A) day 7 and (B) day 14. (a) Hyperemia, (b) fatty degeneration, (c) inflammatory cell infiltration, (d) necrosis (hepatocyte cells), (e) edema, (f) sinusoids (disappeared), and (g) sinusoids. Magnification: 400×.

Figure 6 shows the *Z. mauritiana* Lam. treatment group. The hepatocyte cells are arranged radially towards the central vein. In the control group, the liver's defense system to respond to antigens has changed. Figure 7 shows the response of the liver as depicted by the presence of inflammatory cell infiltration, formation of hyperemia, and fat degeneration. This finding means that the *Z. mauritiana* Lam. treatment group can provide good protection and prevent the spread of *C. albicans* infection. The azole treatment group had minimal protection against the liver, and the liver gave a strong response to prevent *C. albicans* infection activity.

No	Compound	Bioactivity					
		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	(Cyanomethyl) cyclopentane	−3.07	−2.94	−3.5	−3.37	−3.01	−2.84
2	Neophytadiene	−0.12	−0.02	−0.35	0.20	−0.11	0.14
3	Phytol	0.11	0.16	−0.32	0.35	0.00	0.31
4	Hexadecanoic acid	−0.04	0.05	−0.42	0.01	−0.11	0.16
5	Phytol	0.11	0.16	−0.32	0.35	0.00	0.31
6	Linolenic acid	0.33	0.23	−0.19	0.35	0.13	0.42
7	Octadecanoic acid	0.11	0.05	−0.20	0.17	0.06	0.20
8	hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.21	−0.01	0.00	0.12	0.14	0.27
9	13-Octadecenal	0.08	0.26	−0.22	0.04	0.11	0.30
10	Octadecanoic acid, 2,3-dihydroxypropyl ester	0.17	0.04	−0.04	0.12	0.15	0.22
11	Squalen	0.04	0.01	−0.10	0.19	−0.03	0.16
12	Farnesol	−0.13	0.22	−0.60	0.20	−0.43	0.42
13	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	0.09	0.05	−0.09	0.46	0.03	0.34
14	Vitamin E	0.25	0.14	−0.21	0.41	0.28	0.24

Table 3. Bioactivity score of *Z. mauritiana* Lam. compounds.

Table 3 shows the physiological activity of several *Z. mauritiana* Lam. compounds. The activity involved in the interaction mechanism with GPCR ligands and nuclear receptor ligands, and it inhibited the proteases and other enzymes of *C. albicans* from preventing host cell damage. The bioactivity scores suggest that the most

promising compounds of *Z. mauritiana* Lam. as listed in Table 3 are compound nos. 8, 9, 10 and 14. A number of these compounds may act in more than three mechanisms to prevent the infection and development of *C. albicans*. This insight is based on the value of the bioactivity score. If the value is larger than .00, then it is more likely to indicate considerable biological activity; if the value is between −0.50 to 0.00, then it is only partly active; if the score less than −0.50, then it is inactive [23].

The examination of antioxidant quality with respect to the inhibition concentration 50 (IC50) value indicates that *Z. mauritiana* Lam. has a strong free-radical scavenging power. In principle, the less active ingredients needed to reduce DPPH, the stronger the antioxidant quality. The IC50 value of *Z. mauritiana* Lam. is 1.9 ppm, whereas that of the standard vitamin C is 6.21 ppm. The antioxidant power for all tested concentrations was significantly different (p <.05:.000).

Discussion

The results of this study showed that *Z. mauritiana* Lam. can immunologically prevent mucosal infection by *C. albicans* and affect the response of the blood metabolic system, including the immune cell response and blood electrolyte activity. In addition, *Z. mauritiana* Lam. can histopathologically control and help liver metabolism to increase the infection response by facilitating the response of blood immune cells against *C. albicans*. An increase in Candida infection can weaken the immune system. In addition, candida species were reported as the most common fungi infecting the liver. This fungal infection is most common in autoimmune diseases treated with chemotherapy[6].

Abdallah (2016) reported that *Z. mauritiana* Lam. contains several bioactive phytochemical constituents, such as saponins, tannins, alkaloids, phenolic compounds, terpenoids, and flavonoids. A number of these compounds act as antibacterial and antioxidant. In addition, this natural ingredient has a strong antioxidant quantity (IC50 value of 0.024 g/L), which can reduce inflammation by up to 71.83% (400 mg/kg body weight of rats) [24]. The results of this study are in line with the quality of antioxidants in the previous work, i.e., the IC50 value of 1.8 ppm based on the DPPH test.

As previously shown in Figure 1, the healing process of mucosal infections are similar between the treatment group and the azole group. Both groups have the effect of accelerating the healing of infection, as indicated by the thickening of the keratin. The epithelial cells of the buccal mucosa are also relatively neat and regular. Furthermore, less inflammatory cell infiltration and relatively dense connective tissues are observed. Dense/fibrous connective tissue has a matrix containing numerous collagen fibers that play a role in wound healing or defects due to infection. Johnson (2014) reported that oral mucosal wound healing can be characterized by a much more reduced rate of infection or reduced scarring, a faster rate of re-epithelialization, a lower level of inflammation, and a lower rate of angiogenesis compared with wound repair in the skin[25].

Wound healing is a complex cellular process that involves restoring the integrity of tissue damage during infection[26]. *Z. mauritiana* Lam. and azoles work pharmacodynamically and pharmacokinetically by increasing cellular activities, such as migration, proliferation, adhesion, and differentiation to improve wound healing. In addition, several medicinal plants can increase the body's protein metabolism system to supply nutrients to the injured area, thereby helping to accelerate the wound healing process[27]. Figure 2 shows the histopathology of mucosal tissues filled with *C. albicans* blastospore cells. In line with these results, Table 1 has previously shown that the population of *C. albicans* is decreased after being given the *Z. mauritiana* Lam. ethanol extract and azoles. This decrease indicates that *Z. mauritiana* Lam. as a natural antifungal and azole as a synthetic antifungal have similar properties to prevent the spread of *C. albicans* infection to the oral mucosa. *Z. mauritiana* Lam. as a medicinal plant may work by disrupting cell membranes, subsequently influencing the active and passive diffusion activities on the cell membrane surface [28]. Moreover, *Z. mauritiana* Lam. increases membrane permeability by decreasing the hydrophobicity of the cell surface [29]. Meanwhile, azoles work by inhibiting the P450 lanosterol 14-alpha-demethylase enzyme. This enzyme converts lanosterol into ergosterol; the depletion of ergosterol can damage fungal cell membranes, resulting in cell death [30]. Azoles are

synthetic and semi-synthetic compounds with a broad spectral activity [31].

Figures 3 and 4 show the percentage of blood immune cells that respond to infection against *C. albicans*. Both of these test materials can increase the role of blood immune cells. However, *Z. mauritiana* Lam. can reduce the role of lymphocyte antibody cells. Nonetheless, the differences in response to infection against *C. albicans* were non-significant. On day 14, the role of neutrophils has decreased, and they are taken over by lymphocytes. Lymphocytes function as part of the immune system, and it consists of ß cells, T cells, and natural killer cells [32].

Nicholson (2016) reported that neutrophils are a type of white blood cell that works to help fight infection while protecting it from disease threats. White blood cells play a major role in the immune system [33]. The results showed that, on day 7, the monocytes were 5% in the azole group. Meanwhile, on day 14, both groups experienced a decrease in monocyte cells, each at 2%. Monocytes can help to address *C. albicans* fungal infection. Monocytes are involved early in infection control, and they are more effective in killing *C. albicans* compared with dendritic cells or macrophages [34]. The presence of monocytes as a form of response to inflammation triggered by the *C. albicans* infection helped to prevent infection, including removing damaging cells and tissues, and it increased the body's immunity to antigens [4]. In addition, an excessively high amount of monocytes is another important indicator of the development of infection [35]. In this study, the monocyte count had a low percentage (2%) on day 14, and it was 5% on day 7 of the infection treatment.

On day 14, 14% of eosinophil cells were found in the azole group. Certainly, the *C. albicans* infection in the oral mucosa of *R. norvegicus* (experimental animals) has triggered infection and allergy. The allergic response by eosinophils shows a mild infection phase. Thus, *Z. mauritiana* Lam. and azoles may play a role in reducing *C. albicans* infection. Brown (2012) reported that monocytes circulate in the blood at a plasma ratio of 3% to 5% for one to three days, and then they migrate to all body tissues. Once in the tissue, monocytes mature and differentiate into macrophages, dendritic cells, and osteoclasts [36].

The response of immune cells to *C. albicans* is shown in Figure 5, in which antibodies were found in the blood serum. The role of antibodies in the serum was beyond attracting, opsonizing, and phagocytizing. The two test materials used in this study presented a highly similar ability to induce antibody responses against *C. albicans*. In the early infection phase (day 7) the role of antibodies (immunoglobulin M) is possible. However, at a relatively long period (e.g., more than 15 days), if infection persists, then IgG is more dominant than IgM [37]. IgM activates a complement system to bind to the antigen. Meanwhile, IgG antibodies usually have a high affinity, and they are found in the extracellular fluid of the blood [38]. These antibodies, in addition to neutralizing toxins, viruses, bacteria, and fungi, undergo an opsonization process and are phagocytized, subsequently activating the complement system [39]. The active compounds contained in plant extracts act as antioxidants and immunostimulators to increase antibody work, including the work of IgM and IgG when responding to infection with antigens [40].

The results previously presented in Table 2 show the varying blood electrolyte values between the azole groups and between the treatment groups. The difference is an indicator of the occurrence of blood immune cell responses to *C. albicans*. According to the results of the study, the potassium value increased on day 7 for the two groups. By contrast, on day 14, only the azole group experienced an increase, whereas the treatment group with *Z. mauritiana* Lam. ethanol extract decreased with respect to the normal blood potassium level (3.5–5.5 mmol/L). This finding means that, on day 14, the *Z. mauritiana* Lam. test material was able to inhibit the development of *C. albicans* infection, thereby reducing the response of blood immune cells. Potassium is one of the most important minerals in the body that helps regulate fluid balance, muscle contraction, and nerve signals. Its existence is extremely necessary when the body has an infection [41]. The presence of high potassium in the blood is closely related to the role of several bioactivity elements, including the function of ion channel modulators, nuclear receptor ligands, and GPCR ligands. A number of these ligands bind to potassium to maintain water retention and help lower blood pressure, thereby preventing the development of pathogens in the blood [42].

The bioactivity of *Z. mauritiana* Lam. previously shown in Table 3 is correlated with its biological ability to repair infected mucosal tissue, reduce the frequency of *C. albicans*, and improve liver metabolism to increase blood and tissue antibody responses. In general, the bioactivity value of all ligands of *Z. mauritiana* Lam. compounds has a strong ability to interact with antigen receptors. In addition, *Z. mauritiana* Lam. is involved in the mechanism and interaction with GPCR ligands and nuclear receptor ligands, and it inhibits proteases and other enzymes from *C. albicans* to prevent host cell damage. In general, the bioactivity score of *Z. mauritiana* Lam. is between −0.50 to 0.00, whereas some compounds are higher than 0.00. This difference means that the compounds contained in *Z. mauritiana* Lam. have an active biological activity [43].

The trends previously shown in Figures 6 and 7 indicate that *Z. mauritiana* Lam. has a good response to the involvement of liver against *C. albicans* infection in the oral mucosa. Histopathologically, hepatocyte cells began to be arranged radially, i.e., towards the central vein. Meanwhile, inflammatory cell infiltration, hyperemia, and fat degeneration were found in the azole group. In general, this infection is acute, as no connective tissue is found in liver histopathology. After this phase, healing will occur in line with the healing of ulcers on the mucosa. Robinson (2016) reported that, in an infected liver, the function of tissue regeneration and healing metabolism is required in the inflammatory phase because this inflammation creates the potential for immune activation [44]. Hence, the liver immune system can tolerate harmless molecules while remaining alert against possible infectious agents, malignant cells, or tissue damage.

Conclusion

The bioactivity and antioxidant qualities of *Z. mauritiana* Lam. can help to reduce oral mucosal infections and prevent the development of *C. albicans* while providing a biological balance of immune cell and blood electrolyte responses to the development of *C. albicans* infection. In addition, *Z. mauritiana* Lam. can maintain liver cell metabolism when an inflammatory response occurs because it offers a certain active biological activity from GPCR ligands, nuclear receptor ligands, protease inhibitors, and enzyme inhibitors.

A cknowledgements

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Institutional Board Review Statement: The ethical aspects of this study were approved by the Ethical Committee of the Faculty of Dentistry, Syiah Kuala University, Darussalam Banda Aceh Indonesia (no. 2017/KE/FKG/2020). The treatment of experimental animals in this study used the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines released by the National Center for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) guidelines [45], by adopted protocol animal welfare (Farm Animal Welfare Council, United Kingdom) and provided the appropriate facility [46].

Declaration of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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