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In Silico: Analysis of the Potential of Bromelain, Zingibain and Papain as an Alternative Medicine for Fungal Keratitis

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ABSTRACT

Background: Fungal keratitis is an acute or chronic inflammation of the cornea caused by fungi. The main treatment for fungal keratitis is Natamycin 5%. Disadvantages of natamycin are its poor penetration and increased resistance due to fungi which lower the level of ergosterol in its wall.
Purpose: Investigates the effect of bromelain, zingibain and papain on LOX-1 receptors in fungal keratitis caused by Aspergillus fumigatus.
Result: The LOX-1 receptor with bromelain produces a binding energy value of -827.8. While the results of docking LOX-1 receptors with zingibain resulted in a binding energy value of -1096.3. Furthermore, the results of docking the LOX-1 receptor with papain resulted in a binding energy value of -900.0.
Conclusion: zingibain has the highest potency among the three enzymes Papain as an Alternative Medicine for Fungal Keratitis

KEYWORD: Fungi, Fungal Keratitis, Bromelain, Zingibain, Papain

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INTRODUCTION

Fungal keratitis is an acute or chronic inflammation of the cornea caused by fungi (Goel Insan et al., 2013). According to the World Health Organization (WHO), fungal keratitis is a neglected tropical disease with the second highest incidence after bacterial keratitis according to the Asia Cornea Society Infectious Keratitis Study (ACS IKS). The number of new cases of fungal keratitis infection annually is 1,000,000 cases, with a blindness rate of 25% (Niu et al., 2020).

Fungal keratitis infection is caused by Aspergillus spp and Fusarium spp in countries with tropical and sub-tropical climates (Brown et al., 2021). Based on a comparative study comparing fungal keratitis caused by Aspergillus spp and Fusarium spp, it was found that infection with Aspergillus spp fungal keratitis had a treatment failure rate of 31% and was more prone to perforation when compared to bacterial keratitis (Lalitha et al., 2006). Among Aspergillus species, Aspergillus fumigatus is the most common pathogen causing corneal ulceration (Sarika et al., 2021). The main treatment for fungal keratitis is Natamycin 5% which belongs to the azole group. The way it works is to target the ergosterol synthesis pathway, namely by inhibiting 14α-lanosterol dimethylase. Disadvantages of natamycin are its poor penetration and increased resistance due to fungi which lower the level of ergosterol in its wall. In addition, until now there is no treatment for fungal keratitis that can remove the scar tissue that has formed (Hoffman et al., 2021; Raj et al., 2021).

Chitin, -glucan and mannanwhich is found in the fungal cell wall functions as a PAMP which will be recognized by pattern recognition receptors (PRR). C-type lectin receptors (CLR), Toll-like receptors (TLR), nucleotide-binding oligomerization domain-like receptors (NOD-NLRs), and scavenger receptors (SR) are the main PRRs that work in fungal recognition and the body's immune response. LOX-1 is part of the SR that has a CLR-like structure that can be found on endothelial cells, macrophages, neutrophils, platelets, and vascular smooth muscle cells, and human corneal epithelial cells (Gao et al., 2016; Jiang et al., 2019) . During infection with Aspergillus fumigatus, there will be an upregulation of LOX-1 expression.

The bromelain enzyme comes from a species of pineapple named Ananas comosus which is part of the Bromeliaceae family. Bromelain is composed of a complex mixture of proteases and non-proteases (Abreu and De Figueiredo, 2019). Proteases have an important role in the regulation of protein synthesis and degradation, control of

proliferation, the digestive system, as well as the replication and spread of bacteria, viruses, fungi, and parasites (Hikisz and Bernasinska-Slomczewska, 2021). Bromelain enzymes derived from pineapple stems can survive at temperatures of 40-50 C where many enzymes have started to denature (Chakraborty et al., 2021). In previous in vitro studies, bromelain and trypsin administration significantly increased phagocytosis and killed Candida albicans (Rathnavelu et al., 2016; Hikisz and Bernasinska-Slomczewska, 2021).

The zingibain enzymes derived from ginger shows high proteolytic activity (Nafi et al., 2013). Ginger (Zingiber officinale) comes from Asia Pacific which is spread from India to China. North Sumatra, Bengkulu, West Java, Central Java and East Java are ginger centers in Indonesia (Prima et al., 2017).

The papain enzymes obtained from Carica papaya which is a tropical succulent plant and herbaceous plant that has independent stems that grow in all tropical countries and many subtropical regions of the world. In addition, papaya is also available every year because there is no limit due to the season. The papain enzyme is a strong proteolytic and is stable even on denaturing agents. The papain enzyme also has high specificity so that it will not inhibit the proteolysis process in healthy tissues (Amri and Mamboya, 2012).

Currently, there is no molecular docking study that investigates the effect of bromelain, zingibain and papain on LOX-1 receptors in fungal keratitis caused by Aspergillus fumigatus.

MATERIALS AND METHODS

Materials and tools

In this study, two URLs are used, namely, a protein data bank that functions to search for protein or enzyme codes and a docking tool to perform the docking process.

No	Function	URL
1	Protein Data Bank	https://www.rcsb.org/
2	Docking Tool	https://cluspro.bu.edu/

Preparation of Ligand and Receptor Structures

Protein Code Data Bank (PDB) structure of papain enzyme (9PAP), zingibain enzyme (1CQD), bromelain enzyme (1BI6) and receptorsLOX-1 (1YPQ) is obtained by accessing the site https://www.rcsb.org/. The following are pictures of the structure of the three enzymes that will be used for docking:

No	Enzyme Name	Structure
1	Bromelain	
2	Zingibain	
3	Papain	

Enzyme Ligand Docking Simulation

The code obtained is then used for the molecular docking process on the http://cluspro.bu.edu/ site. The PDB codes for papain, zingibain and bromelain enzymes are then entered in the ligand column while the codeLOX-1 (1YPQ) was used to fill the receptor column. After the docking process was carried out for approximately 4 hours, various Binding Interaction Models (MAP) and binding energy were obtained for each interaction between the receptor and the ligand.

Analysis of docking data from the Cluspro application based on the value of binding energy. The molecule with the lowest binding energy value shows a stable interaction so that it has the potential to be used as a proteolytic. Therefore, the interaction model that has the lowest binding energy is the best.

RESULT

Test Ligand Screening

Characteristics	Bromelain (1BI6)	Zingibain (1CQD)	Papain (9PAP)
Structure Weight	5.89 kDa	99.14 kDa	24.43 kDa
Number of Atoms	403	7.171	1,908
Residual Amount	52	864	212
Unique Protein Chain	2	1	1

The table above shows the characteristics of the three enzymes used for docking, namely bromelain, zingibain and papain.

Enzyme Ligand Docking Simulation

The results of the docking simulation between the LOX-1 receptor and the ligands of the three enzymes are depicted in Figure below.

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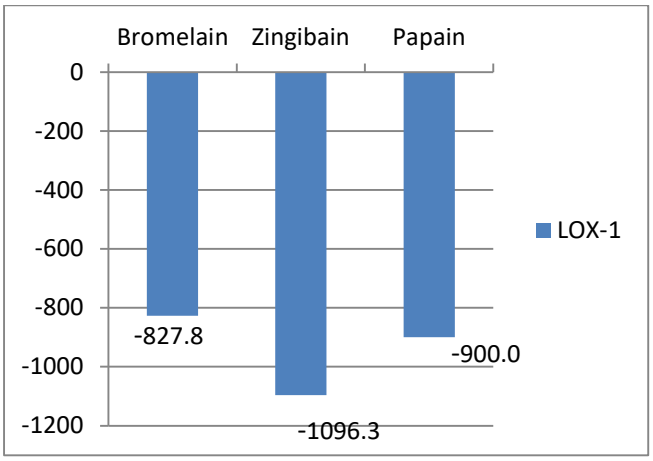
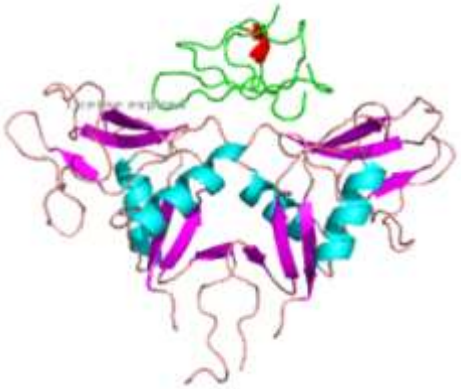
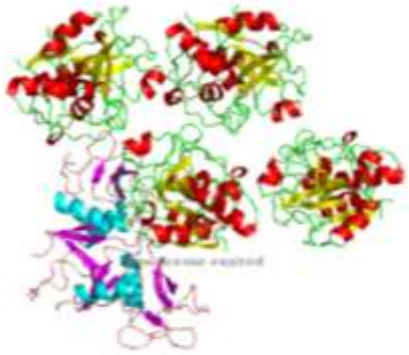


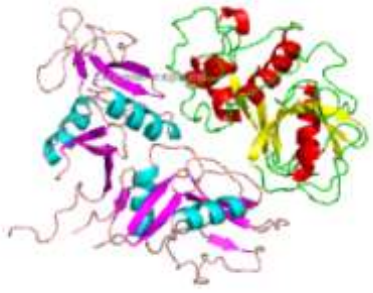
It can be seen in the graph above, the LOX-1 receptor with bromelain produces a binding energy value of -827.8. While the results of docking LOX-1 receptors with zingibain resulted in a binding energy value of -1096.3. Furthermore, the results of docking the LOX-1 receptor with papain resulted in a binding energy value of -900.0.



Bromelain withLOX-1



Zingibain withLOX-1



Papain with LOX-1

ANOVA Test

This ANOVA test uses the SPSS application, the following are the results:

Test of Homogeneity of Variances				
	Levene Statistic	df1	df2	Sig.
Bromelain	2,070	2	27	,146
Zingibain	1,441	2	27	,254
Papain	5,332	2	27	,011

Results of variances homogenity test show that all groups are appropriate for anova test because have value sig. > 0.01

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Bromelain	Between Groups	10671,715	2	5335,857	6,282	,006
	Within Groups	22932,775	27	849,362		
	Total	33604,490	29			
Zingibain	Between Groups	70427,552	2	35213,776	26,012	,000
	Within Groups	36551,220	27	1353,749		
	Total	106978,772	29			
Papain	Between Groups	28488,339	2	14244,169	9,640	,001
	Within Groups	39896,260	27	1477,639		
	Total	68384,599	29			

Anova test results

All groups have significant differences (sig. < 0.01)

DISCUSSION

Keratitis is a group of acute or chronic inflammatory disorders of the cornea caused by disruption of the protective mechanism of the outer layer of the eye (Ahmadikia et al., 2021). Keratitis can be categorized into several types based on the causative pathogen, namely bacteria, fungi, viruses, and protozoa (Recent trends: Medical management). Keratomycosis or fungal keratitis is a fungal infection of the cornea caused by a broad spectrum of fungi and yeasts (Mahmoudi et al., 2018). As many as 25% of patients with fungal keratitis may lose their vision. According to the Asia Cornea Society Infectious Keratitis Study (ACS IKS) fungal keratitis is one of the second most common microbial keratitis after bacterial keratitis (Kuo et al., 2022), and is a neglected tropical disease according to the World Health Organization (WHO) (Ahmadikia et al. , 2021). In addition, the incidence of fungal keratitis continues to increase, especially in developing countries (Mills et al., 2021). Additional factors that exacerbate the burden of the disease in developing

countries include a lack of population awareness about the disease and a lack of medical resources. These factors lead to poor outcomes due to misdiagnosis, delayed or inappropriate treatment (Sadik et al., 2022).

There are more than 100 species that cause infection, but Aspergillus spp., Fusarium spp., and Candida spp., are the causative agents of 95% of cases of fungal keratitis (Mahmoudi et al., 2018). Infections caused by Fonsecaea pedrosoi, Cylindrocarpon sp, Paecilomyces sp, Scedosporium prolificans, Lasiodiplodia theobromae, Pythium insidiosum, and Metarhizium anisopliae are less frequently reported. (Niu et al., 2020). In sub-tropical and tropical areas the majority of infections are caused by fungi, with Aspergillus spp and Fusarium spp as the main species, while in temperate climates it is mostly caused by yeast with Candida spp. is the main causative agent (Brown et al., 2021). Thus, geographical differences indicate the prevalence of different causes of fungal keratitis (Chongkae et al., 2021). Based on a comparative study comparing fungal keratitis caused by Aspergillus spp and Fusarium spp, it was found that infection with Aspergillus spp fungal keratitis had a treatment failure rate of 31% and was more prone to perforation when compared to bacterial keratitis (Lalitha et al., 2006). Among Aspergillus species, Aspergillus fumigatus is the most common pathogen causing corneal ulceration (Sarika et al., 2021).

Fungal keratitis often results from minor ocular trauma (Brown et al., 2021). This is because the eye will become more susceptible to pathogens after epithelial injury (Kuo et al., 2022). Based on a study at a tertiary health service in Thailand which reviewed 294 patients with fungal keratitis for 8 years and found that trauma was the most common risk factor (Chongkae et al., 2021). The use of contact lenses, especially for long periods of time, the use of insufficient disinfectant solutions, trauma, eye surgery, especially corneal surgery are the most common predisposing factors (Lakhundi et al., 2017). In addition, work is also said to be with fungal keratitis. Workers who come into contact with soil, such as agro-industry and construction workers, are reported to be more susceptible to corneal infections. This is due to traumatic agents present in plants, animals, and even dust (Brown et al., 2021). Not only that, a history of prolonged use of contact lenses, corneal surgery, especially penetrating keratoplasty, and the use of antibiotics due to the development of broad-spectrum antibiotics and steroids can also make a person more susceptible to infection with keratomycosis. Diabetes mellitus, cancer, HIV infection, and use of drugs that can cause systemic immunosuppression can also be associated with fungal keratitis (Bourcier et al., 2017). In developed countries such as Australia, ocular surface disease and use of contact lenses, as well as HIV infection in America are major risk factors for corneal infection.

The prevalence of fungal keratitis is higher in agricultural areas with warm and humid climates. The frequency of positive corneal cultures is in the range of 20% to 60% (Ahmadikia et al., 2021). Fungal keratitis has 1,000,000 new cases of infection each year with a high rate of blindness (Niu et al., 2020). A multi-center prospective study in Taiwan found a higher rate of fungal identification in the tropics compared to the subtropics. Temperatures in the tropics and sub-tropics are on average higher than 18 °C in winter, but winter monthly rainfall is much less in the tropics. Leck et al. found a higher incidence of fungal keratitis during dry and windy seasons than rainy and humid seasons (Chen et al., 2020). Based on observations in India it was found that fungal keratitis cases peaked during the months of high agricultural activity according to the rice sowing in July and the harvest season in January (Ghosh et al., 2016). In line with previous studies both in southern Thailand and other countries, male fungal keratitis patients were seen more often than women in a ratio of 3:1 (Chongkae et al., 2021). Patients with fungal keratitis are dominated by men with most cases found in the age group of 21-60 years (Ghosh et al., 2016). In Indonesia, the incidence of keratitis and corneal ulcers in 1993 was 5.3 per 100,000 population in Indonesia, the ratio of men and women was not very significant in the incidence of keratitis research in Hospital.

The development of fungal keratitis is generally slower than that of bacterial keratitis. The course of infection depends on the virulence of the fungus, the size of the inoculum, and the resistance of the host. The first clinical manifestations appear several hours or days after trauma or wearing infected contact lenses, but may appear later. Based on a study on clinical signs of fungal keratitis conducted by Ibrahim et al. 42.1% of patients had hypopyon, 36.8% of patients had satellite infiltrates, 13.2% had ring infiltrates. The location of the most infiltrates was found in the central part of 57.9% of patients. Furthermore, in paracentral as many as 36.8% of patients and then in the periphery as much as 5.3%. As for the depth of the infiltrates, the majority were found in the deep section, namely 71.1% of patients and superficial as many as 28. 9% of patients (Harbiyeli et al., 2022). Another clinical sign that may indicate fungal keratitis is a grayish-colored corneal epithelium with a surface without ulceration or infiltration. The epithelium is sometimes found intact, healing over an extensive stromal infiltrate, endothelial plaque, and Wessely's immune ring. (Leck and Burton, 2015; (Ahmadikia et al., 2021). Aspergillus or Fusarium infection can progress very quickly to corneal perforation and endophthalmitis, especially after corticosteroid therapy (Bourcier et al., 2017). Clinical manifestations and medical history can help. diagnosis, but these characteristics are less clear in comparison and can mimic the characteristics of both bacterial and parasitic keratitis (Ahmadikia et al., 2021; Tananuvat et al., 2021).Especially in the early stages of keratitis, where corneal suppuration may mask the clinical characteristics of fungal keratitis (Chen et al., 2020). Therefore history and clinical clues have a lower value in making the correct diagnosis for FK when compared to bacterial keratitis (Tannuvat et al., 2021).

Fungal keratitis is more complicated and challenging than bacterial keratitis due to the less predictable susceptibility of fungi to antifungal agents. A previous study of 31% of fungal keratitis patients did not respond to initial treatment. This is worse when compared to bacterial keratitis (Tannuvat et al., 2021). The drug of choice for the majority of filamentous fungal keratitis is 5% natamycin suspension, although penetration into the corneal stroma is poor and the drug is difficult to formulate. The way it works is by binding to sterols, especially ergosterol, which is on the fungal cell membrane which will result in instability and death of the fungus (Austin et al., 2017; Hoffman et al., 2021; Raj et al., 2021).

Alternative medicine thatTopical amphotericin B can be given is 0.3% to 0.5%. Amphotericin B is a broad-spectrum antifungal polyene macrolide produced by Actinomyces Streptomyces nodusus and was the first antifungal agent to be used clinically but its use is limited due to its toxicity. In addition, Voriconazole, which belongs to the broad-spectrum Azole group, can be administered as drops of 0.1% or 1% or topical tablets of 200 mg which have perfect ocular penetration and oral administration shows good ocular improvement. Voriconazole works by inhibiting 14 a-lanosterol dimethylase which consequently affects ergosterol synthesis. Ergosterol is an important component of fungal cell walls. Voriconazole is often given to patients who fail to treat with natamycin and amphotericin B.

*Mycotic ulcer treatment trial*I (MUTT I) was a randomized controlled clinical trial comparing topical natamycin and topical voriconazole in the treatment of filamentous fungal ulcers showing the superiority of natamycin over voriconazole which was demonstrated by better visual acuity and lower perforation rate. This difference was more pronounced in Fusarium ulceration, where best corrected visual acuity (BSCVA) of four lines, three-month-old lesions were also found to be smaller on natamycin therapy. The percentage of patients with positive culture results after six days of treatment was also higher with the use of voriconazole. This also proves that natamycin is better for the treatment of all fungal species. (Solanki et al., 2015; Austin et al., 2017). Based on cohort research, fungal keratitis caused by Aspergillus spp. treated with natamycin had a higher failure rate, whereas fungal keratitis caused by Fusarium spp was less susceptible to voriconazole (Kuo et al., 2022).

With proper management, the decreased vision caused by infection and inflammation of the cornea can improve, but the patient's vision will still be worse than his pre-infection vision. This is because there is no treatment that can remove the scar tissue that has formed. In addition, many patients whose condition worsened after being treated with 5% natamycin. Therefore, it is necessary to find new alternative treatments in the treatment of fungal keratitis (Hoffman et al., 2021).

Ananas comosus is the name of a species of pineapple, which belongs to the family Bromeliaceae (Pavan et al., 2012; Abreu and De Figueiredo, 2019). Pineapple can be found in various tropical and subtropical countries, such as China, the Philippines, Kenya, India, Indonesia, Thailand, and Malaysia (Pavan et al., 2012). Pineapple is a major source of bromelain, even in its inedible part (Abreu and De Figueiredo, 2019). Bromelain is a proteolytic enzyme that contains one sulfhydryl group and has a function as a proteinase inhibitor. Therefore bromelain is considered to have good therapeutic potential (Chakraborty et al., 2021).

Ginger, known botanically as Zingiber officinale, is native to South Asia but has spread to many other regions of the world. Long ago, ginger was used as medicine in India, China and Europe. Today, ginger is one of the most important and widely used spices in the world. Ginger is also used in the pharmaceutical field because of the presence of phenolic substances gingerol and shagaol in the rhizome which are reported to have anti-cancer and antioxidant activities (Ghasemzadeh et al., 2010).

The ginger protease or zingibain, which was first reported as a novel protease source in 1973, exhibits remarkable proteolytic activity. Zingibain is a meat tenderizer that is highly active against collagen and other connective tissue proteins. Good milk coagulation activity is also associated with zingibain so it is used in the preparation of ginger milk curd in southern China (Nafi et al., 2014). Zingibain can be accessed with the code 1CQD on PDB.

Papain is a plant endolytic cysteine protease enzyme isolated from papaya latex (Carica papaya L.). Papain is obtained by cutting the latex raw papaya skin that flows from the incision then collected and dried. The greener the fruit, the more active papain. The papain enzyme belongs to the papain superfamily, as a proteolytic enzyme, papain is essential in many vital biological processes in all living organisms. Papain exhibits extensive proteolytic activity against proteins, short chain peptides, amino acid esters and amide links and is widely applied in food and medicine fields. Papain preferentially cleaves peptide bonds involving basic amino acids, especially arginine, lysine and residues after phenylalanine (Amri and Mamboya, 2012).

CONCLUSION

From the research that has been done, the docking between LOX-1 and zingibain has the smallest binding energy value of -1096.3 compared to papain which is worth -900.0 and bromelain which is worth -827.8. So zingibain has the highest potency among the three enzymes Papain as an Alternative Medicine for Fungal Keratitis.

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