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The Activity of Bromelain Enzyme from Pineapple (*Ananas Comosus* (L) Merr): A Review

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Abstract:
Background:Bromelain is a protein-digesting enzyme that can accelerate a hydrolysis reaction of protein. This enzyme has a form like a yellowish-white powder. To determine how to isolate this enzyme, it is first necessary to know the location of the enzyme in the source. Furthermore, similar types of enzymes from different sources will have different activity values. The purpose of this literature review is to conduct a literature search on the activity of the bromelain enzyme contained in pineapple (*Ananas Comosus* (L) Merr) in the pharmaceutical sector.
Materials and Methods: The method used is the collection of library research data that has been published through search engines such as Google Scholar, PubMed, and ScienceDirect.
Results: Various studies have shown that bromelain from pineapple peel extract has antibacterial activity that can inhibit the growth of *Streptococcus mutants* and *Enterococcus faecalis*. Furthermore, this pineapple peel extract has antifungal activity against the growth of *Pityrosporum ovale* and *Candida albicans*. Then the pineapple fruit extract has an anti-inflammatory effect on osteoarthritis patients. Then the pineapple peel extract has a coagulation effect that can prolong the bleeding time, and finally, the pineapple weevil extract can induce apoptosis in squamous carcinoma cell cultures.
Conclusion: Thus the bromelain in pineapple has various activities in the pharmaceutical field.
Key Word:anti-aggregationt, antibacterial, anti-inflammatory, antifungal, anticancer, bromelain, extract pineapple

I. INTRODUCTION

An enzyme is a form of substance that acts as a catalyst, derived from living cell organisms to regulate the rate of a chemical reaction without involving the reaction. The application of this enzyme has been widely carried out in industrial fields, such as in food products, agriculture, chemicals, and pharmaceuticals[(1)]. One example of an enzyme is the enzyme bromelain. Bromelain belongs to the group of proteins that have strong bonds with other compounds in the vicinity. Bromelain is a protein-digesting enzyme or protease enzyme that can accelerate a hydrolysis reaction of protein. This enzyme has a form like a yellowish-white powder[(2)].
Several research results show that bromelain can be used in the pharmaceutical field, including: antibacterial against *Streptococcus mutans*[(3)]; antibacterial against *Enterococcus faecalis*[(4)]; antifungal *Pityrosporum ovale* and *Candida albicans*[(5)]; anti-inflammatory in osteoarthritis patients[(6)]; Has a coagulation effect that can prolong bleeding time[(7)]; and can induce apoptosis of tongue squamous carcinoma cell cultures in humans[(8)]. Bromelain enzymes can also be used in the food industry. Utilization of this enzyme can be applied; baking soda to produce a more fluffy dough; meat tenderizer; anti-browning to inhibit fruit browning and phenol oxidation; manufacture of alcoholic beverages; and to regulate beer stability and reduce foaming[(9)]. Bromelain enzymes are widely applied in the pharmaceutical and food fields. Therefore we need a way to isolate this enzyme. First, it is necessary to determine the location of the enzyme in the source. The next thing to note is that similar types of enzymes come from different sources, so the value of their enzyme activity will be different. Finally, it is necessary to know the time required for the isolation of this enzyme to be used in activity tests with different sizes[(10)].

The isolated bromelain enzyme was then characterized. The value of the bromelain enzyme isolated from pineapple has an activity unit of 5.373 U/ml, the protein content of 10.299 mg/ml, and the specific activity of 0.521 U/mg[(11)]. The results of the isolation of the bromelain enzyme from pineapple peel were obtained at a temperature of 65^{o}C at 0.071 units/minute and a pH of 6.5 at 0.101 units/minute[(12)]. The results of enzyme isolation from pineapple weevil obtained a pH of 7.0 with an enzyme activity of 1.081 units/g[(13)]. In this review article, we will discuss the activity of the bromelain enzyme contained in pineapple (*Ananas Comosus* (L) Merr) in the pharmaceutical field.

II. MATERIAL AND METHODS

1.Literature Review Search Strategy Design

The design of this research is in the form of references from previous research results based on topics that are by the literature review of articles published in National and International journals that have been accredited or indexed by search engines such as *Google Scholar*, *PubMed*, and *ScienceDirect* using in English, Anti-Aggregation, Anti-bacterial, Anti-inflammatory, Anti-fungal, Anti-cancer, Bromeolin, and Pineapple extract.

2. Criteria for Literature Review

Criteria Literature review is a process for selecting literature based on criteria related to research objectives. The criteria for this literature are seen based on the title, abstract, and keywords. Then the search results that will be reviewed are filtered again by looking at the completeness of the text. This section also uses scientific articles published in the last ten years. From the search results obtained references as many as 65 scientific articles which were then selected as many as 42 scientific articles.

Table no 1: Literature findings

Data Based	Literature Findings	Selected Literature
Google Scholar	**32**	**22**
PubMed	**11**	**7**
ScienceDirect	**22**	**7**
Total	**65**	**36**

III. RESULT

The results of this review article describe a summary of the bromelain enzyme activity from various sources.

Table no 2: Results of research on the activity of the bromelain enzyme in pineapple (*Ananas Comosus* (L) Merr).

Research Purpose	Results	Referensi
To determine the antibacterial properties of pineapple peel extract against *Enterococcus faecalis*	This pineapple peel extract contains bromelain as an antibacterial	(4)
To determine the antifungal in pineapple peel extract against *Pityrosporum ovale* and *Candida albicans*	Pineapple peel extract has an active ingredient, namely bromelain as an antifungal against *Pityrosporum ovale* and *Candida albicans.*	(5)
To determine the anti-inflammatory effect in Osteoarthritis patients	Administration of bromelain content in arthritis patients states that there is an anti-inflammatory effect	(6)
To determine the pineapple peel extract on the coagulation effect	This pineapple peel extract contains bromelain which can be used as an alternative to the coagulation effect which can prolong bleeding and blood clotting time	(7)
To determine the pineapple weevil extract against apoptosis in carcinoma cell culture	This pineapple weevil extract can induce apoptosis due to the presence of bromelain	(8)

IV. DISCUSSION

Based on the research that has been done, it is stated that pineapple contains a fairly high bromelain content. Based on the literature, this bromelain enzyme comes from a plant in the Bromeliaceae family. Isolation of bromelain enzyme was carried out using the maceration extraction method. This method is one of the extraction methods by immersing *simplicia* and not undergoing heating at all by using an appropriate solvent.

Maceration is carried out to extract the active substance contained in pineapple, namely the bromelain enzyme[14]. It should be noted that to obtain enzymes that have good activity, look at the main source of pineapple parts that can meet the requirements, such as pH, enzyme concentration, substrate, temperature, determination of molecular weight, and enzyme structure to determine the functional group at the active site of the enzyme. During the process, the temperature is maintained so that it does not exceed 50^{o}C if the temperature is above 50^{o}C then the work of this enzyme will begin to decrease because the enzyme is a protein which if it is at a high enough temperature it will experience denaturation so that the enzyme obtained is damaged and its activity decreases[15]. Furthermore, the addition of a buffer solution with a pH of 7.0 needs to be considered because at a pH of 7.0 the enzyme has the most suitable conformation with its substrate so that it can form the right substrate enzyme complex and produce maximum activity [16]. At the time of blending which is a physical extraction. Then centrifugation was carried out and the result was a precipitate that served to separate the crude enzyme from the remains of the pineapple tissue and the supernatant in the form of the crude enzyme bromelain. This crude extract generally has low activity because this enzyme is still mixed with several other enzymes that may contain compounds other than the bromelain enzyme. Furthermore, to carry out purification, namely the crude fraction of this bromelain enzyme, it is necessary to add ammonium sulfate with the required level, each fraction is precipitated in 0.1 M phosphate buffer solution pH 7.0 and each fraction is dialyzed[17].

Activity of bromelain

The many benefits of bromelain in everyday life. This discussion will focus on the use of this enzyme in the pharmaceutical field. Based on several studies related to bromelain enzyme activity, including:

1. Antibacterial activity of bromelain

Bromelain enzyme has antibacterial activity against several types of test bacteria. Research result [4] conducting tests using the activity test method it uses is liquid dilution. The concentrations carried out were 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%. Then the number of bacterial colonies was calculated to determine the Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (MBC) against *Enterococcus faecalis*. The results showed that pineapple peel extract (*Ananas Comosus* (L) Merr) had antibacterial activity against the growth of *Enterococcus faecalis* in vitro. This study stated that the MIC value of pineapple peel extract against *Enterecoccus faecalis* was 3.125% and the MBC value was 6.25%. This is due to the content of bromelain in pineapple peel extract. Bromelain has antibacterial properties that are effective against both aerobic and anaerobic strains of bacteria. Bromelain also has antiadhesion properties that can prevent the attachment of bacterial glycoprotein receptors to the intestinal mucosa. Then the substances in the bromelain enzyme can change the physical and chemical properties of cell membranes and block the normal function of bacteria so that they can inhibit and kill these bacteria.

Similar results were also reported by [3] through the good method and it is proven by the presence of obstacle activity in the form of a clear zone surrounding the wellbore. The following are the results of the research conducted by the examiners showing that this pineapple (*Ananas Comosus* (L) Merr) extract has the enzyme bromelain as an antibacterial against the growth of *Streptococcus mutans* (the cause of dental caries) with inhibition zones at concentrations of 25%, 50%, 75%, and 100%. At a concentration of 25%, there is an inhibition zone with an average of 3 mm. Then at concentrations of 50%, 75% and 100% the clear zone was getting bigger. That is, at a concentration of 50% the average is 10 mm, 75% with an average of 12.5 mm. Then at 100% concentration, the average is 20 mm. The effectiveness found in the results of this study was at a concentration of 50% with an average of 10 mm. This shows that the greater the concentration is given, the more the inhibition of bacteria will increase. The content in pineapple which is an antibacterial substance is bromelain. The way bromelain works is by inhibiting the enzyme reverse transcriptase and DNA opoisomerase so that bacterial cells cannot be formed. Bromelain enzyme has antibacterial activity related to its ability to interfere with protein transport in the inner layer of cells. The bromelain enzyme also has a target on cell wall polypeptides so that the formation of cell walls becomes less than perfect. This causes the bacterial cell to lyse due to osmotic pressure so that the bacterial cell will die.

2. Antifungal activity of bromelain

The research results of [5] conducted an activity test using the agar diffusion method with the paper disc technique. The concentrations used are 5%, 10%, and 15%. The results of the inhibition zone testing on *Pityrosporum ovale* and *Candida albicans* showed that pineapple peel extract (*Ananas Comosus* (L) Merr) at a concentration of 15% had an antifungal activity that was effective against *Pityrosporum ovale* and *Candida albicans* compared to concentrations of 5% and 10%. The antifungal potential of pineapple peel is due to the presence of active compounds, one of which is bromelain. Bromelain has antifungal activity by reducing the surface tension of the sterol membrane of the dermatophyte cell wall by increasing its permeability and causing the fungus to die.

The research of [18] conducted an activity test using the dilution method. The concentrations carried out were 100%, 50%, 25%, 12.5%, 6.25%, 3.125% and 1.56%. The results show that the concentration of 6.25% is the MIC value and 12.5% is the MBC value, namely the minimum concentration of pineapple fruit extract that can kill the fungus *Candida albicans*. This proves that the growth of *Candida albicans* colonies is inhibited because of the active compound contained in the pineapple fruit extract, namely bromelain. Bromelain works as an antifungal because it is a sulfhydryl protease enzyme capable of hydrolyzing peptide bonds in fungal cell proteins or polypeptides into smaller molecules, namely amino acids. This causes the lysis of *Candida albicans* cells.

3. Anti-inflammatory activity of bromelain

Results [6] conducted a clinical trial to identify that bromelain used in the daily dose range of 200-2000 mg, had a therapeutic effect at a dose of 160 mg/day. This test aims to assess the effect of the enzyme bromelain on osteoarthritis in the legs by using the enzyme bromelain at a higher therapeutic dose, which is in the range of 540-1890 mg/day. The results of the data obtained that bromelain at this dose for osteoarthritis showed results that were as effective as using medications (NSAIDs) with the same safety and tolerability.

Other research results from [19] bromelain has been recommended as adjuvant therapy for the treatment of chronic inflammatory, malignant, and autoimmune diseases. In vitro studies have shown that bromelain has the ability to modulate receptor molecules on T cells, macrophages, and natural killer cells. This bromelain will activate natural killer cells by increasing the production of stimulatory factors and decreasing the activation of T-helper cells. Thus, that bromelain works by reducing most of the inflammatory mediators.

4. Anti-aggregationt activity of bromelain

The results of research by [7] conducted a platelet anti-aggregation test with an average time made. Bleeding time was measured by injuring the tail of a male white rat. The results showed that group I (28 mg/200 g BW) had an average time of 48.6 seconds. Group II (56 mg/200 g BW) had an average time of 66 seconds. Group III (112 mg/200 g BW) had an average time of 105 seconds. Thus, the dose that has the best effect as an increase in clotting time is group III, because it has the largest difference in blood clotting time, which is 105 seconds. Pineapple peel in group III had the best effect because it contained the highest bromelain enzyme so that the dry squeezed pineapple skin at a dose of 112 mg/200 g BW had a greater increase in activity than the other dose groups. The results of this antiplatelet aggregation test proved that pineapple peel was able to prolong bleeding and blood clotting time in white male rats, due to the presence of bromelain. Bromelain works by breaking down fibrinogen which can cause blood to clot and can also prevent blood clots.

The results of another study,[20] conducted a test of anti-platelet aggregation using bleeding time in rat experimental animals. The results showed that the test group was given the ethanolic extract of pineapple at a dose of 50 mg/kg BW, a dose of 100 mg/Kg BW, a dose of 200 mg/Kg BW, a dose of 300 mg/Kg BW, and a dose of 400 mg/Kg BW. At a dose of 400 mg/Kg, BW showed significant results on the effect of bleeding time because in this treatment pineapple extract contained the bromelain enzyme. The way bromelain works is by the inhibitory effect of thromboxane A2 (TXA2) which can reduce the production of thromboxane so that the platelet aggregation process becomes prolonged.

5. Anticancer activity of bromelain

The research results of [8] conducted a cytotoxicity test to determine the presence or absence of inhibition of growth of human tongue squamous carcinoma cell culture by pineapple weevil extract in various concentrations. The results of this cytotoxicity test were used to obtain the value of Inhibitory Concentration 50 (IC50) of pineapple weevil extract on the percentage of squamous carcinoma cell death of the human tongue. The IC50 value was obtained by using the probit analysis test. Probit analysis was carried out to determine the level of concentration of the material (pineapple hump extract) on the sample response (percentage of cell death). The results showed that pineapple weevil extract had a cytotoxic effect on human tongue squamous carcinoma cell cultures with an IC50 value of 6,324,49 g/mL. In this study, the signal that causes apoptosis is a signal from outside the cell by treatment using pineapple weevil ethanol extract. The ability to induce apoptosis of pineapple weevil ethanol extract is probably due to the presence of the bromelain enzyme in it.

The results of this study are by the research of[21] which states that bromelain can inhibit the proliferation and differentiation of tumor cells in vitro in leukemia cells through an apoptotic mechanism. According to [22], Apoptosis of human tongue squamous carcinoma cell cultures due to the bromelain enzyme can occur through the intrinsic and extrinsic pathways because it can increase the regulation of p53, Bax, caspase 3, and caspase 9, as well as decrease the anti-apoptotic protein bcl2. Based on the description above, the inhibitory effect of pineapple weevil ethanol extract on human tongue squamous carcinoma cell culture can be in the form of decreasing cell proliferation (anti-proliferation) or induction of apoptosis.

In the same study that researched the transcription of gene expression, [23] tested the anticancer activity based on the IC50 value, which is half the maximum inhibitory concentration in cancer cells. Bromelain exerted an inhibitory effect on the growth of MCF-7 cells after 96 hours with an IC50 of 60 g/mL. Then according to [24], the IC50 value which can inhibit the growth of MCF-7 cancer cells was obtained at a concentration of 65 g/mL. Meanwhile[25] obtained the results of the cytotoxic activity of commercial and recombinant bromelain determined using the sulforhodamine (SRB) test where recombinant bromelain and commercial bromelain can affect decreasing the viability of MCF-7 cells with IC50 values of 5 each, 13 and 6.25 g/mL. From the three studies that have been conducted, there is one difference in bromelain concentration that can inhibit 50% of MCF-7 cancer cells. The use of commercial and recombinant bromelain requires lower concentrations to inhibit the growth of MCF-7 cancer cells than pineapple bromelain extract. According to [26], the cytotoxic activity of extracts that attack cancer cells can be classified into three categories. The first category is said to be very active if it has an IC50 value of <10 g/mL, the second category is active if the IC50 is 10-100 g/mL, and the third category is quite active if the IC50 is between 100-500 g/mL. Thus, commercial and recombinant pineapple bromelain has cytotoxic activity against MCF-7 cancer cells with a different activity.

This study aims to determine the mechanism that occurs in bromelain when it inhibits the growth of breast tumor cells. The process of inhibition occurs through increased expression of p53 and Bax, as well as decreased expression of Cox-2 and Bcl-2. This process indicates the occurrence of apoptosis. Compounds used for cancer therapy can cause DNA damage in cells that result in apoptosis through the p53 pathway [27]. Apoptosis is also an active process by inducing genes such as Bax and simultaneous suppression of genes such as Bcl-2 [28]. This is supported by [29] that Bax apoptotic activation will form a mitochondrial apoptosis-induced channel (MAC) and mediate the release of cytochrome-c, while anti-apoptotic Bcl-2 will block this process.

V. CONCLUSION

Based on the results of a literature review, shows that pineapple contains bromelain which can be used as an antibacterial, antifungal, anticancer, anti-inflammatory, and anti-aggregation.

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Soni Muhsinin, et. al. "The Activity of Bromelain Enzyme from Pineapple (*Ananas comosus* (L) Merr): A Review."*IOSR Journal of Pharmacy (IOSRPHR)*, 11(06), 2021, pp. 27-32.