

Bromelain Enzyme from Pineapple Fruit as an Antiviral Agent Against HIV, Hepatitis C and Human Papiloma Virus

¹Marulip Andjaitan, ²Tutunnugraha, ¹Kezia Hendardi Pamudja, ¹Firmanp Idris,
¹Meliana Tjandra and ¹Tiffany Wiriantono
¹Department of Biomedical Engineering
²Department of Pharmaceutical Engineering, Swiss German University,
BSD City, Banten, Indonesia

Abstract: The objective of this research was to investigate the potential use of bromelain enzyme, a proteolytic enzyme contained in the flesh and stem of a pineapple fruit, as antiviral agent against HIV (Human Immunodeficiency Virus) that causes AIDS (Acquired Immuno Deficiency Syndrome), Hepatitis C Virus (HCV), and Human Papiloma Virus (HPV) that causes cervical cancer. The investigation include *in vitro* studies to see the interactions of the enzyme and the viruses. Blood samples containing HIV, HCV and HPV Virus were contacted with solutions that contained bromelain enzyme. Effects to the virus were tested using several methods including test strip methods, DNA concentrations in the solutions as the viral envelop was damaged, as well as Scanning Electron Microscopy (SEM) technique. Additionally, a cooperativework was done with a clinic in the Northern part of Jakarta and with a low priority correctional facility in Bogor (West Java) that were coping with patients with HIV/AIDS. In this resaech, the patients were provided with 2 glasses of fresh pineapple juice every day. The changes in CD4+ and blood HIV viral loadwere followed during these trials. The outcomes have shown that bromelain enzyme have the potential to be utilized as antiviral agent. The resaech relied on the possibility that bromelain enzyme could cause some damages on viral envelopes that has protein as one of its structural component. As this viral envelope was damaged buy the proteolytic acitivies, the virus had either lost its capability to reproduce or was destroyed altogether, since, the viral outer layer was damaged. These positive effects of bromelain activities towards these viruses have been shown *in-vitro* in our lab. Bromelain enzyme originating from the flesh and the stem of pineapple fruit has been shown to have the potential to be utilized as antiviral agent againstviral borne diseases which include HIV/AIDS, Hepatitis C and HPV that could cause cervical cancer. The enzyme utilized in the treatment of the patients can be relatively easily obtained by extracting the juice of pineapple fruit or by directly consuming the juice as has been described in this.

Key words: HIV, AIDS, hepatitis virus, HPV virus, antiviral, bromelain, pineapple

INTRODUCTION

Bromelain is a proteolytic enzyme found naturally in pineapples (*Ananascomosus*) from the Bromeliaceae family. The enzyme is a family of sulfhydryl-containing proteolytic enzymes that also contains a peroxidase, acid phosphatase, several protease inhibitors and organically bound calcium (Kelly, 1996). The enzyme bromelain functions as a catalyst in the hydrolysis or breakdown of peptide bonds of proteins. The enzyme is contained within the edible flesh of the fruit but especially, richer within the hardy stem at the core of the fruit. The initial works carried out for some pineapple varieties available in Indonesia showed that for the locally available pineapple

fruit, 65% of the enzyme activity was found within the stem while 35% was contained within the flesh (Carolina, 2009). Thus, throughout the work, the importance of the inclusion of the fruit stem was always emphasized.

Various past studies have shown that bromelain possesses various healing qualities. As digestive aid for example, bromelain can help the digestion process in the case of pancreatotomy, exocrine pancreas insufficiency and other intestinal disorders (Kelly, 1996). Bromelain is also known to be able to modulate immunological responses (Engwerda *et al.*, 2001). Others also discussed its function as anti-inflammatory agents, that can be used in rheumatoid arthritis, thrombophlebitis, hematomas, oral inflammations, diabetic ulcers, rectal and

perirectal inflammations as well as athletic injuries, oral and plastic surgery (Kelly, 1996; Taussig and Batkin, 1988).

In this research, the potential of bromelain enzyme as an antiviral agent was studied which focused on infections due to HIV (Human Immunodeficiency Syndrome) virus that causes AIDS (Acquired Immuno Deficiency Syndrome), HCV (Hepatitis C Virus) and HPV (Human Papiloma Virus) virus that can cause cervical cancer in women. This study summarizes some of the results obtained during this investigation. Furthermore, the impact of consuming bromelain enzyme that was contained in fresh pineapple juice on patients with HIV/AIDS were also tested in two separate studies. One involved 7 HIV/AIDS patients under the care of a clinic in Jakarta while the second one was involving 11 patients in a low priority correctional facility in Bogor, a city in West Java Province located just at the outskirts of Jakarta. For the HPV virus test, blood samples were acquired from a clinic known locally as the Puskesmas Garuda in the city of Bandung, West Java Province, approximately 180 km South of Jakarta. For HCV tests, the blood samples were acquired from 3 patients that were tested positive for the HCV virus from Marzuki Mahdi Hospital (Bogor, West Java).

MATERIALS AND METHODS

Anti HIV/AIDS tests: The HIV virus used in the initial part of this research was in the form of blood serum obtained from five HIV-positive patients from a local clinic in North of Jakarta (Puskesmas Penjarangan). Their blood samples were treated with EDTA and were centrifuged to obtain the serum. During the initial step of this research, the sample serum was incubated at 37°C for 2-4 h at 37°C, with 50 µL of various bromelain solution concentrations. To indicate HIV infection, qualitative analysis was done using Mono HIV (1&2) test strips for serum and plasma.

The purified bromelain was purchased in powder form from Sigma. This bromelain powder was dissolved in phosphate buffer pH 7 to produce different types of bromelain solution of different concentrations and activities. To test the activity of the bromelain powder (Sigma) and bromelain solution, quantitative analysis was used including measurement of total protein and volume activity to further calculate the specific activity of the enzyme. The results showed that all blood samples gave negative results on HIV detection when incubated with bromelain at a concentration of 10 mg/mL (Pamudja, 2009).

A short program was carried out involving 7 HIV patients who with their consent were given 2 glasses/day

of fresh pineapple juice. One of the patients was in the program for a few weeks while the rest were in for a few months. Approximately 300 mL of cold water was added to every batch so the blender knives could run smoothly. The cold water would also protect the activity of the enzyme during the extraction process, particularly protection against the slight warming process that always occurred during blending due to the movement of the blades. Similar to most naturally occurring enzyme, the enzyme extracted here was also highly sensitive to denaturation caused by thermal damage. Furthermore, particular attention was given to the age of the pineapple fruit and the inclusion of both the fruit stem and flesh. It is recommended that not too ripe pineapples were selected, since, they contain higher level of bromelain activities. Sugar may be added to improve the palatability of the juice in case it became too sour. During the test, the immune system cells CD4+ were counted and followed during the course of the experiment. This was done commercially in a private clinic or a government run hospital.

RESULTS AND DISCUSSION

Results of anti HIV/AIDS tests: The results of the first anti HIV/AIDS tests are shown in the following Table 1 (Pandjaitan *et al.*, 2015). Note that, normal level of CD4+ is 410-1590 cell/µL.

In a further test that was done in a closed community, in a low priority correctional facility in the city of Bogor (40 km South of Jakarta), it was found that 12 inmates were HIV positive. The outcome of the test can be seen in Table 2. The results also include viral load tests which provided the number of viral counts/mL. The results had been mixed. Some patients showed some improvement in CD4+ counts while some remained at similar level. The results of the viral load counts were also similar. A method of viral load detection that is more sensitive with lower detection limit is required for future investigations. The project is still on going and further analysis is still being carried out.

This second trial at the correctional facility was also accompanied by kidney function test (creatinin, ureum)

Table 1: Initial test with fresh pineapple juice consumption with 7 patients (2 glasses/day)

Patients No.	CD4+ counts before pineapple juice consumption (cell/uL)	CD4+ counts after pineapple juice consumption (cell/uL*)
1	135	647
2	<200	361
3	<200	723
4	391	688
5	75	199
6	49	221
7	3	129

Table 2: Further test with 12 patients including one control

Patients No.	Initial CD4+ (cell/mL)	CD4+ after 16 weeks of treatment (cell/mL)	Initial viral load (copies/mL)	Viral load after 16 weeks (copies/mL)
1	409	574	Undetected	Undetected
2	425	595	Undetected	Undetected
3	85	-	Undetected	Undetected
4	762	578	277,000	-
5	335	427	4,230	2080
6	598	492	8,420	18900
7	323	-	7,290	16400
8	375	549	Undetected	-
9	491	235	179	Undetected
10	396	362	Undetected	Undetected
11	268	258	Undetected	Undetected
12	430	379	184	Undetected
13 (control)	515	510	Undetected	Undetected

to check if there were any adverse effects of the relatively high intake of bromelain enzyme into the body. The results showed that the level of ureum and creatinin for all of the patients remained within normal range. This showed that the bromelain intake did not interfere with the functionality of kidney within the 16 weeks, during which the test was conducted. Despite the fact that pineapple has been consumed regularly by the population such adverse effects might exist considering the level of consumption was substantially higher compared to regular dosages. Based on the results, however such adverse effects as far as the kidnet function was not detectable.

A further test was also carried out using an electron microscope to see the effects of HIV virus when it was exposed to bromelain enzyme (*in vitro*). The results showed that that there were some morphology changes of the virus after the exposure. From the picture, it can be clearly seen that in the control sample, the viru's morphology which is pointed with arrow have a perfect circle shapes. Whereas in the second picture that shows the treated sample morphology with the same magnification, the virus shape became distorted and in more oval or irregular shapes and also in some of the virus, a tail like object could be seen around the virus particle that was assumed to be the protein channel fracture of the virus that lead to the distortion of the virus. It is likely that the proteolytic nature of the enzyme could cause some damages to the viral envelopes. It is known that the viral envelope also contains proteins which could become susceptible to attack by bromelain enzyme (Fig. 1).

Anti Human Papiloma Virus (HPV) tests: In this part of the investigation, the purpose was to study the potential use of bromelaine enzymes to neutralize the HPV virus. This virus is known to cause cervical cancer in women who had been infected with this virus. Similar with the work on HIV, the utilization of bromelain here was owing

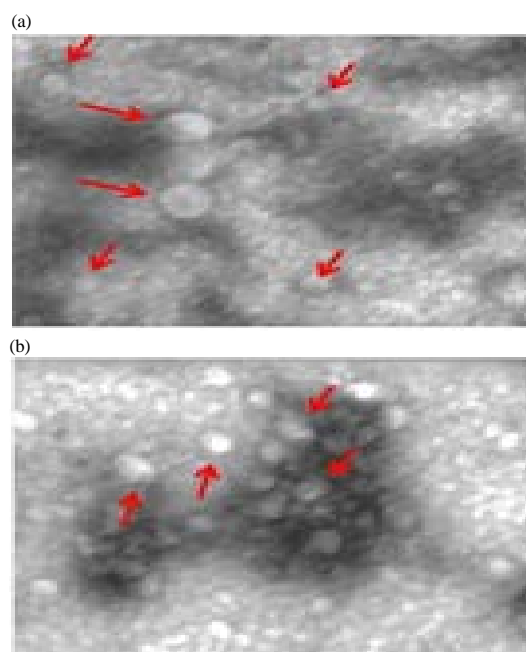


Fig. 1: Electron microscope results of HIV virus before and after exposure to Bromelain enzyme

to the fact that the protease enzyme bromelain was expected to interact with the protein in the outer layer of the viral particles. The capsomers that surrounded the genome of the virus was made up of proteins. Any damage to the protein in the outer layer was expected to cause the DNA of the HPV to come out from within the virus's envelope and subsequently, the DNA would be spilled into the solution and could become detectable in the solution phase. Such process would destroy the virus particles and at the same time, experimentally, this would facilitate the detection of the DNA of the HPV in the solution phase. Thus, by measuring and comparing DNA concentrations in solutions that were originally contained in the HPV particles that were treated with bromelaine and

those that were not could give a clear view of the effectiveness of the enzyme in degrading the virus protective capsid.

During this research, samples containing the HPV virus were exposed to bromelain enzyme of different concentrations and at different duration of incubation time. In order to detect the presence of HPV DNA in the samples that have been collected, PCR (Polymerase Chain Reactions) technique was used. The procedure for detecting HPV DNA itself could be divided into three steps which were the DNA extraction, DNA amplification by PCR and visualization of the PCR results by using gel electrophoresis. During the study, the method was continuously improved to ascertain that the DNA can be detected properly. The detail of the studies and the methodology can be found at Firman (2010). Furthermore, the bromelain enzyme that was utilized in this research was extracted from pineapple fruit. Both the flesh and the stem bromelain were included in the extraction. The detail of method of extraction can also be found in the same reference. The samples containing the HPV virus needed for the analysis were obtained from the local clinic (Puskesmas) Garuda in the city of Bandung, West Java Province, approximately 180 km south of Jakarta.

Results of anti HPV tests: The outcome of the study (Fig. 2) showed that there was a substantial increase in the concentration of nucleic acid following treatment of samples with bromelain enzyme. The concentration of bromelain enzyme that was suitable to give detectable impact was found to be the one with specific activity of 5.9 μmg protein, volume activity of 150.9 μmL and incubation time of 6 h at 37°C. The DNA concentrations in the samples were found to increase by 164.8 and 270.8%.

The increase of the DNA concentrations from both samples was possible because the capsid of the HPV virus and some the membrane of the cell was degraded which allowed, the DNA of the virus and some DNA from the cell to get into the solution tested here. In order to prove that the DNA that leaked out into the sample was from the HPV virus, the PCR technique was applied. After the DNA concentration had been proven to rise in both of the patient's sample, the effect of the enzyme towards the visualization and amplification of the HPV DNA was done. The result of the PCR can be seen in Fig. 3. From the result, it can be summarized that the more HPV DNA concentration that was present in the sample, the more prominent the band that is shown on the agarose gel is. The PCR analysis proves that the DNA concentration that was in the sample contained the HPV DNA that had leaked out of the viral capsid and transferred into the

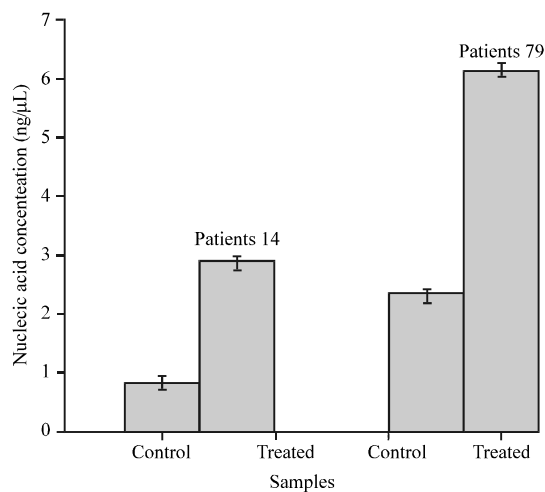


Fig. 2: Changes in nucleic acid concentration after treatment with bromelain, samples were from two different patients

solution. The results of incubation time of 6 h on samples from the two patients (Patients No. 14 and 79) displayed a prominent and clear band that proves that the HPV DNA is available in a higher amount.

Anti Hepatitis-C Virus (HCV) tests: Similar with the case of the HIV and HPV, the use of bromelain on the HCV was based on the fact that the HCV also possesses structure that contains protein at its outer layer. It was also hypothesized that bromelain could act as a possible antiviral to the HCV by destroying the envelope glycoproteins in the outer layer of the HCV particles that helps the virus to reproduce. The HCV positive serums used in this study were obtained from Marzuki Mahdi Hospital (Bogor, West Java). The sample serums came from 3 patients that were tested positive for the HCV virus. The sample serums were evenly divided and placed into microtubes containing 200 μL of serum each. All the serums were then stored in the freezer at -20°C until they were needed for the experiments. The enzyme bromelain used for this research was bought from Sigma-Aldrich Chemie GmbH with a product code of B4882-10G originating from pineapple fruit.

In this research (Wiriantono, 2012), the effects of Bromelain on HCV were investigated using the MONO™ Anti-HCV tests. In a similar manner as the HIV virus tests, the research was done by incubating HCV-positive serums with bromelain solution of various concentrations at 37°C at various time of incubation. The 3 sample serums from 3 different Hepatitis C patients were incubated at 37°C with Bromelain solutions at concentrations of 10, 15 and 20 mg/mL for 1, 2, 4 and 6 h. The experiment was then

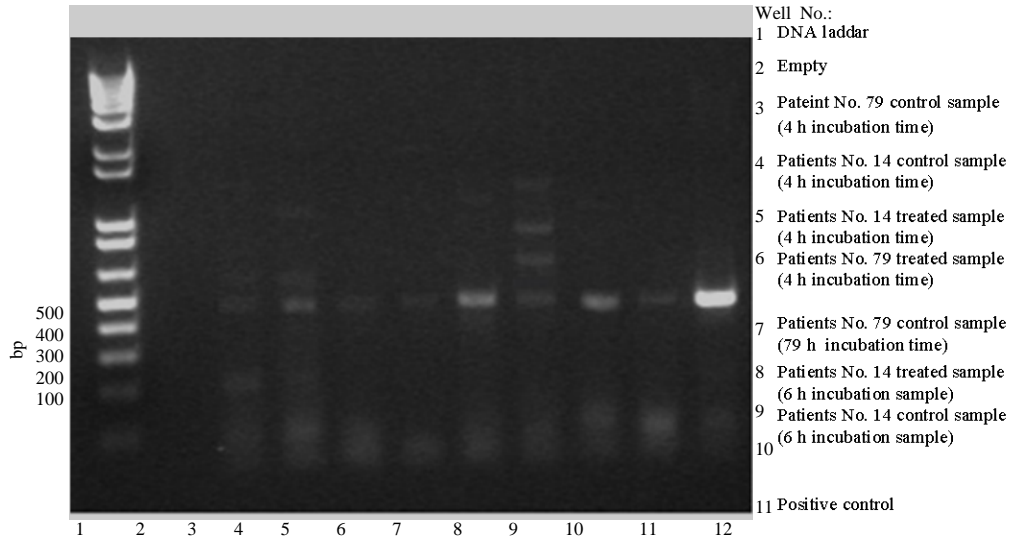


Fig. 3: PCR Analysis of samples containing HPV virus after treatment with bromelain enzyme

Table 3: The results of the mono™ anti HCV tests for blood samples from 3 HCV positive patients

Patients	Incubation time (h)	Bromelain concentration (mg/mL)		
		10	15	20
1	1	Positive	Positive	Negative
1	2	Positive	Positive	Negative
1	4	Positive	Positive	Negative
1	6	Positive	Positive	Negative
2	1	Positive	Positive	Negative
2	2	Positive	Positive	Negative
2	4	Positive	Positive	Negative
2	6	Positive	Positive	Negative
3	1	Positive	Positive	Negative
3	2	Positive	Positive	Negative
3	4	Positive	Positive	Negative
3	6	Positive	Positive	Negative

repeated to a total number of 3 times to ensure the accuracy of the results. A control was also made for each sample serum treated with a certain concentration of bromelain. The controls were made by mixing the HCV positive serum with a pH 7 phosphate buffer instead of with Bromelain solution. The experiment results that were repeated three times were found to be consistently similar (Table 3).

Results of anti HCV tests: The result of the experiment showed that a bromelain concentration of 20 mg/mL with a specific activity of 4.395 μ/mg and an incubation time of 1 h at 37°C were needed to produce a negative test result for the MONO™ Anti-HCV test for all serums. For the control, the sample serums from all 3 patients were also mixed with a pH 7 phosphate buffer instead of bromelain solution, the MONO™ anti-HCV test showed a positive result for all 3 patients and remained positive even

after 1, 2, 4 and 6 h of incubation. This confirms that the negative result was indeed caused by bromelain enzyme.

CONCLUSION

Bromelain enzyme originating from the flesh and the stem of pineapple fruit has been shown to have the potential to be utilized as antiviral agent against virus borne diseases which include HIV/AIDS, Hepatitis C and HPV that could cause cervical cancer. In this research, the investigation include *in vitro* studies to see the interactions of the enzyme and the viruses as well as some direct trials in which fresh pineapple juice was consumed by HIV/AIDS patients. The enzyme utilized in the treatment of the patients can be relatively easily obtained by extracting the juice of pineapple fruit or by directly consuming the juice as has been described in this research.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Central Research Funds (CRF) at Swiss German University as the main source of fundings for this research. Furthermore, our appreciations also were given to the Puskesmas (Clinic) Penjaringan (in North Jakarta), Bogor District Commission for the handling of HIV/AIDS (KPAD), Tangerang District General Hospital (RSUD Tangerang), Marzuki Mahdi Hospital in Bogor (West Java) as well as the Puskemas (clinic) Garuda in the City of Bandung (West Java) for their cooperation. We are also expressing our gratitude to the Government of the City of Bogor for their financial

contribution that had facilitated the initial collaborative study between the Department of Biomedical Engineering, Swiss German University with the KPAD in Bogor.

REFERENCES

- Carolina, V., 2009. Purification of high Grade of Bromela in from Pineapple. BSc Thesis, Swiss German University, Bumi Serpong Damai, Indonesia.
- Engwerda, C.R., D. Andrew, A. Ladhams and T.L. Mynott, 2001. Bromelain modulates T cell and B cell immune responses in vitro and in vivo. *Cell. Immunol.*, 210: 66-75.
- Firman, P.I., 2010. Preliminary study of antiviral for Human Papilloma Virus (HPV) using protease enzyme bromelaine. BSc Thesis, Swiss German University, Tangerang, Indonesia.
- Kelly, G.S., 1996. Bromelain: A literature review and discussion of its therapeutic applications. *Altern. Med. Rev.*, 1: 243-257.
- Pamudja, K.H., 2009. Preliminary study of antiviral for Human Immunodeficiency Virus (HIV) using protease enzyme (Bromelain). BSc Thesis, Swiss German University, Tangerang, Indonesia.
- Pandjaitan, M., M. Tjandra, T. Aswani, F. Ughi and T. Permana *et al.*, 2015. Alternative therapy for human immunodeficiency virus/acquired immunodeficiency syndrome patients using pineapple juice. *Adv. Sci. Eng. Med.*, 7: 911-914.
- Taussig, S.J. and S. Batkin, 1988. Bromelain, the enzyme complex of pineapple (*Ananas comosus*) and its clinical application. An update. *J. Ethnopharmacol.*, 22: 191-203.
- Wiriantono, T., 2012. Preliminary study of antiviral for Hepatitis C Virus (HCV) using protease enzyme (Bromelain). BSc Thesis, Swiss German University, Tangerang, Indonesia.