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EXTRACELLULAR PRODUCTION AND MICROBIAL IDENTIFICATIONOF BROMELIAN ENZYME FROM PINEAPPLE (ANANAS COMOSUS)

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Abstract: - A significant protease that was identified from pineapple (Ananas comosus) is called bromelain. Depending on its source, bromelain accumulates to adifferent degree and has varied qualities throughout the entire plant. Using sodium citrate buffer and Whatman filter paper, bromelain was recovered from the entire pineapple. For 10 minutes, the filtrate was centrifuged at 6000 rpm. This was regarded as a crude enzyme. The aforementioned content underwent a step of dialysis to produce the purified enzyme. A gelatin digestion unit was developed to assess and visualize the crude extract's activity in the hydrolysis of gelatin (GDU). BSA was used as a standard in the Folin-Lowry assay, which was used to estimate the protein concentration. On the basis of various pH, temperature, activator, and inhibitor values, the enzyme's kinetics were estimated. This study's objective was to assess if bromelain, a substance extracted from pineapple waste, could reduce acne because of its numerous antibacterial qualities.

Keywords: - Protease, Activator, Inhibitor, Antioxidant, Antibacterial

A. Introduction: - The pineapple, sometimes referred to by its scientific name Ananas comosus, is a member of the Bromeliaceae plant family. For many years, South America was where it was first grown and was found by Europeans. The

sulfhydryl proteolytic enzymes in bromelain are primarily responsible for its characteristics. Depending on the source of the protease, bromelain is categorized as stem bromelain or fruit bromelain. Bromelain is found throughout the entire pineapple plant, however the amount and makeup might vary based on the part of the fruit and the variety. Any enzyme that

performs proteolysis activity by hydrolyzing the peptide bonds that connect the amino acids in the polypeptide chain creating the protein is referred to as a protease, also known as a peptidase or proteinase. Proteases have undergone several evolutionary cycles, and various protease classes can carry out the same reaction using entirely distinct catalytic pathways. Animals, plants, microbes, archea, and viruses all have proteases in them. As evidenced by their long-standing use as organic "tenderizers" for meat, papaya and pineapple are two of the richest plant sources. The proteolytic enzymes included in these fruits go by the names papain and bromelain, respectively. It has been found to have anti-inflammatory, anti-platelet aggregation, fibrinolytic, blood-thinning, anticancer, and antibacterial properties. Studying the effects of active bromelain on bacterial infections like acne required the isolation of this compound from pineapple waste. It will be more affordable to complete the procedure if bromelain, which is found in pineapple waste, is used.

B. Methodology:-

- **Sample Collection**

The sample was obtained at the Borivali market in Mumbai. Wash in runningwater and prepare the extract for further used with a mortar and pestle

Fig 1:- Crown and leaf of pineapple which contains the enzyme

- **Bromelain extraction from pineapple (Ananas Comsus)**

With the used of a mortar and pestle and 100ml of 0. 1m sodium citrate buffer pH 5, 50gms of pineapple peel was homogenized. This was filtered and centrifuged at 6000 rpm for 10 minutes. The supernatant was used as a crude enzyme. Tris HCl was used to dissolve the pellet

- **Crude extract enzyme assay**

	Gelatin	Crude Enzyme	D/W (ml)	H_2O_2 (µL)	Formaldehyde (ml)	Volume of NaOH run down
Test	2.5	0.1	-	0.1	1.0	7.8
Blank	2.5	-	0.1	0.1	1.0	6.6

- **Obtaining purified enzyme**

1) Ammonium sulphate precipitation: - 44 grams of ammonium sulphate should been weighed. Ammonium sulphate should been added one pinched at a time to the enzyme suspension. For 30 minutes, shook the contents. Held it there overnight at 4 °c. Tris HCl was used to dissolve the pellet

2) Dialysis: - Distilled water that was boiling had 2% sodium carbonate added and 10 cm of dialysis membrane was allowed to soak for 30 minutes. Carefully filling the tube with the sample solution up to two-thirds of the way, the opened end was then tied off with a thread to seal it. The packagewas inserted into the sodium phosphate buffer

a) **Folin-Lowry Protein Estimation Method: -** The protease mixtures was estimated for the protein content by used Folin-Lowry assay. Bovine serum albumin (BSA) was used as a standard at 1 mg/ml. Freshly prepared alkaline copper sulphate solution ((50:1) 2% na2co3 in 0. 1 N NaOH: 0.5% $CuSO_4$in 1% NaK), Folin-Ciocalteu phenol reagent (FC) 1 N was used. 1 ml of protein was added to 5.5 ml of alkaline copper solution and incubated for 10 min. 0.5 ml of fc reagent was added to the mixture and incubated in dark for 30 min and absorbance was recorded (Plummer and Plummer, 1988)

b) **Effect of Different pH on Enzyme Activity: -** Each test tube received 2.5ml of the gelatin solution, which was subsequently kept at the various pH values of 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 All test tubes except the blank received 1ml of enzyme, which was then incubated at 45 °c for 10 minutes. To each test tube that was empty, 0.01 microliter of hydrogen peroxide was introduced along with 0. 1 ml of distilled watered.

After used 0.05 N NaOH got the pH down to 6.9, 1.0 ml of formaldehyde was added to each test tube. Titration was carried out until 0.05 N NaOH produced a pH 7.8 and the NaOH volume was depleted

c) **Effect of Different temperature on Enzyme Activity: -** Each test tube received 2.5ml of the gelatin solution, which was subsequently kept at the various temperature values of 35, 40, 45, 50, 55, 65, 75 and 85°c. All test tubes except the blank received 1ml of enzyme, which was then incubated at 45°c for 10 minutes. To each test tube that was empty, 0.01 microliter of hydrogen peroxide was introduced along with 0.1 ml of distilled watered. After used 0.05 N NaOH got the pH down to 6.9, 1.0 ml of formaldehyde was added to each test tube. Titration was carried out until 0.05 N NaOH produced a pH 7.8 and the NaOH volume was depleted

d) **Enzyme activity and the effect of the activator: -** Each test tube received 2.5 ml of the gelatin solution before the $MgCl_2$ inhibitor was kept in a separate volume 10-20-40-60-100 microliters. Every test tube, excluding the blank one, received 0.1ml of enzyme, which was then incubated at 45 °c for 10 minutes. In addition to 0.01 microliter of hydrogen peroxide, 0.1 ml of distilled watered was supplied to each test tube. Added 1.0 ml of formaldehyde to each test tube after adjusting the pH to 6.9 with 0.05 N NaOH. Titration was carried out until 0.05 N NaOH produced a pH reading of 7.8 and the remaining NaOH was exhausted.

e) **Enzyme activity and the effect of the inhibitor: -** Each test tube received 2.5 ml of the gelatin solution before the $HgCl_2$ inhibitor was kept in a separate volume 10-20-40-60-100 microliters. Every test tube, excluding the blank one, received 0.1ml of enzyme, which was then incubated at 45 °c for 10 minutes. In addition to 0.01 microliter of hydrogen peroxide, 0. 1 ml of distilled watered was supplied to each test tube. Added 1.0 ml of formaldehyde to each test tube after adjusting the pH to 6.9 with 0.05 n NaOH. Titration was carried out until 0.05 N NaOH produced a pH reading of 7.8 and the remaining NaOH was exhausted.

f) **DPPH-based assay for antioxidant activity: -** The antioxidant activity of the extracts and standards was based on the 2, 2 diphenyl-1-picrylhydrazyl's (DPPH) capacity to scavenge free radicals. At a 1:1 ratio, the test sample/standard was combined with DPPH and incubated for 30 min in the dark. The mixes of solution was read at 517 nm. The blank was ethanol withDPPH (1:1)

- **Antibacterial activity: -** The fruit's juice, which includes the enzyme, was tested for presence of Staphylococcus aureus and Bacillus subtilis. This was accomplished using the agar cup method, which revealed a zone of inhibition all around, indicating the antibacterial activity's mechanism.

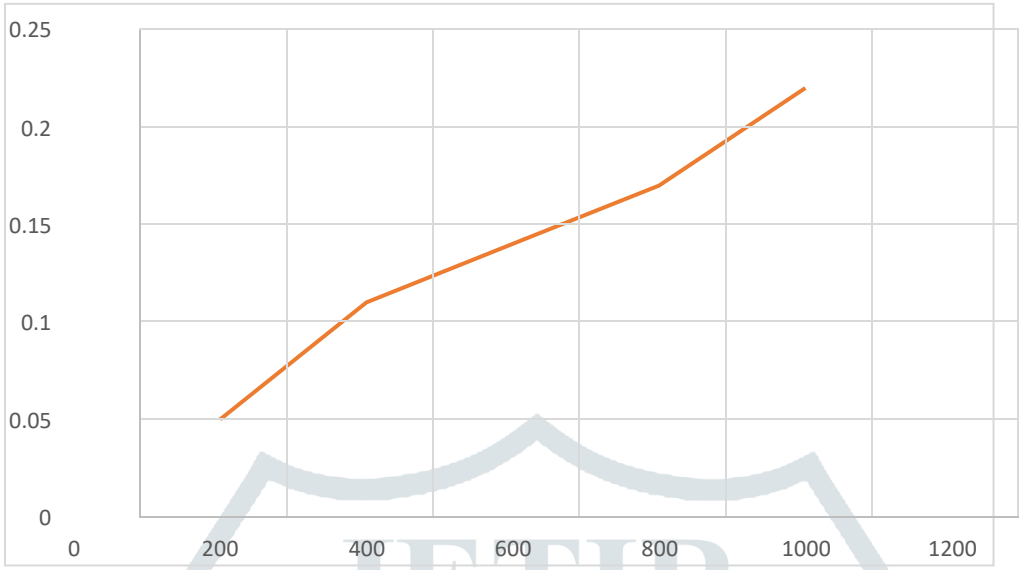
C) Results:-

- **Enzyme Assay for Crude extract**

Enzyme Activity = (Volume of test – Volume of sample) (N)
14 X 100 /Mg enzyme/RM …. (4)
=7.8-6.6 X 0.1 X 14 X 1000/0.6
=2625 units/mg

➢ The enzyme activity was found to be 2625 units/mg.

a) Folin-Lowry Protein Estimation Method:-



Graph 1:- Estimation of Protein Content by Folin-Lowry Method

Concentration of protein was found to be 0.64mg/ml

b) Table 1:- Effect of Different pH on Enzyme Activity

pH	Volume of NaOH run down (ml)
3.5	1.6
4.0	1.7
4.5	1.9
5.0	2.6
5.5	4.0
6.0	4.8
6.5	5.9
7.0	7.0
7.5	6.2
8.0	5.9
8.5	5.6

c) Table 2:- Effect of Different temperature on Enzyme Activity

Temperature	Volume of NaOH run down (ml)
35°C	1.9
45°C	2.7
55°C	3.6
65°C	5.2
75°C	3.9
85°C	2.0

Table 3:- Enzyme activity and the effect of the activator

$MgCl_2$ activator	Volume of NaOH run down (ml)
10 µL	0.8
20 µL	1.6
40 µL	2.2
60 µL	2.6
100 µL	3.1

Table 4:- Enzyme activity and the effect of thr in inhitor

$HgCl_2$ inhibitor	Volume of NaOH run down (ml)
10 µL	0.9
20 µL	2.1
40 µL	1.6
60 µL	0.5
100 µL	0.1

From the above 4 tables, following results were found:-

Table 1:- The optimum pH was found to be pH 7

Table 2:- The optimum temperature was found to be 65°C

Table 3:- The activator ($MgCl_2$) concentration of 100µL was determined tomaximise the enzyme activity.

Table 4:- The maximum enzyme activity decreased after the inhibitor concentration reached 40µL.

d) DPPH-based assay for antioxidant activity:-

% inhibition= Control – Test/ Control X 100

= 1.19-0.82/1.19 X 100

= 31% inhibition

- The antioxidant activity showed 31% inhibition.

- **Specific activity**

Measured using following formula

Specific activity =Enzyme activity/protein concentration ….. (4)

- Hence the specific activity was found to be 4101.56 units/mg

- **Antibacterial activity:-**

Fig 2 (a) shows the activity towards Staphylococcus aureus and Fig 2 (b) shows towards Bacillus subtilis. Zones of inhibition measured were 22mm for fig 2 (a) and 13mm, 26mm and 15mm respectively for 1, 3 and 4 respectively. This indicates that the enzyme shows antibacterial activity towards the two organisms.

D) Discussion: - A family of proteolytic enzymes derived from Ananas comosus isknown as bromelain and includes members that contain sulfhydryl. A sulfhydryl proteolytic fraction makes up the majority of bromelain. Moreover, it includes calcium that is organically bonded, peroxides, acid phosphatase, and many protease inhibitors. It is a cysteine endopeptidase that preferentially cleaves peptidebonds at the carbonyl group as found in arginine or in aromatic amino acids like phenylalanine or tyrosine. The juice was referred to as a crude extract of the enzymes, and the amount of activity in these crude extracts was determined by hydrolyzing gelatin and reported as gelatin digestion units (GDUs). Several factorswere investigated, including pH, temperature, activator, and inhibitor. With the aid of the agar cup method, the antibacterial activity revealed zones of inhibition.

E) Acknowledgement:-

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