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ANTI-MICROBIAL POTENTIALS OF PINEAPPLE & KIWI FRUIT

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***Abstract*— The enzyme present in kiwi that is analogous to bromelain in pineapple is actinidain. Actinidain is a cysteine protease enzyme that plays a crucial role in kiwi fruit ripening and softening by enzymatically breaking down proteins. This enzyme exhibits a broad substrate specificity and optimal activity at slightly acidic pH levels. Actinidain has also been shown to possess various biological activities, including anti-inflammatory, anti-cancer, and digestive aid properties. Understanding the properties and functions of actinidain in kiwi fruit not only contributes to our knowledge of fruit ripening processes but also has potential implications for various industrial and health applications.**

This dissertation provides a comprehensive comparison of two widely studied proteolytic enzymes, bromelain and actinidin, in terms of their biochemical properties, physiological functions, and potential therapeutic applications. Bromelain, derived from pineapple, and actinidin, found in kiwifruit, are both cysteine proteases that exhibit significant proteolytic activity. The review covers their diverse enzymatic properties, including substrate specificities, optimal pH and temperature ranges, and stability profiles.

Furthermore, the dissertation delves into the various physiological roles of bromelain and actinidin in food digestion, anti-inflammatory effects, wound healing, and immune modulation based on a thorough analysis of existing research studies.

In addition, the dissertation sheds light on the emerging therapeutic applications of bromelain and actinidin in clinical settings, including their potential for cancer therapy, anti-bacterial activity, and modulation of allergic responses. By comparing the biochemical properties, physiological functions, and therapeutic potentials of bromelain and actinidin, this dissertation provides valuable insights for researchers, clinicians, and industry professionals working in the field of enzymology and biotechnology.

Overall, this review elucidates the distinct characteristics of bromelain and actinidin, highlighting their similarities and differences in terms of enzymatic activities, structural features, physiological functions, and therapeutic applications, thereby contributing to a deeper understanding of the potential uses of these proteolytic enzymes in diverse fields of science and medicine.

***Index Terms*— Kiwi & Pineapple fruit, well diffusion method, Gram Positive & Gram Negative bacteria, antimicrobial, potential.**

I. INTRODUCTION

Pineapple and kiwi both possess antibiotic properties due to their high content of bromelain and actinidain enzymes, respectively. Bromelain in pineapple exhibits anti-inflammatory and anti-bacterial effects, while actinidain in kiwi aids in digestion and strengthens the immune system. These enzymes help in combating harmful bacteria and reducing inflammation in the body.

Moreover, both fruits are rich in vitamin C, which is essential for supporting the immune system and promoting overall health. The combination of these unique properties makes pineapple and kiwi ideal natural sources of antibiotic benefits.

Incorporating pineapple and kiwi into your diet can be a flavorful way to boost your immune system and support your body's natural defenses against infection, way to harness their antibiotic properties.

Types of fruits:

1. Type 1: Pineapples with crowns attached to fruit.

2. Type 2: Pineapples without crown attached to fruit.

3. Type 1: kiwi fruit

II. LITERATURE REVIEW

Holyavka M *et.al.,* still, for bromelain transfer, there's presently no standard, largely effective immobilization system.[1] Ataide *et.al.,* with chitosan offered physical protection, crack retraction, and delayed release, all of which are desirable in topical phrasings with a modified release. It's possible to reduce the carrier's influence on the structural as well as functional [3].

Pineapple plant has chief protease enzymes in it known as Bromelain [2], it was chemically found in year 1879[5]. It was firstly identified in 1891 by Marcano [6].Bromelain was investigation and isolate in year 1894[7]. it was firstly introduced in to medicinal supplemented in year 1957[5],[7].

kiwi peel along with its pulp were dissolved within water which has concentration of 10mg/ml to identify the antimicrobial activity . In addition, this antibacterial activity of kiwi pulp and peel was assessed through previously used methods Soković *et al.,* (2010) kiwifruit peel also has commercial potential while production of the citric acid and alcohol, kiwi fruit peel is used in the process of solid state fermentation Fattouch *et. al.,* (2008) [8]

III. METHODOLOGY

Collection process

There are 2 type of fruit used in this project Kiwifruit, Actinidia deliciosa & pineapple, Ananas comosus.

This fruit, Mature Kiwifruit & Mature pineapples was collected from cotton market, the famous market Nagpur, Maharashtra. These fruit was washed with tap water and distilled water. peeled and rinsed with tap water and distilled water again to remove the dust particle form it.

The different part of fruit, which was waste like pineapple (crown, peel, and eye)& Kiwifruit (peel and pulp), also preserved to test different concentration of enzyme in different parts. It was kept in the refrigerator till experimental studies.

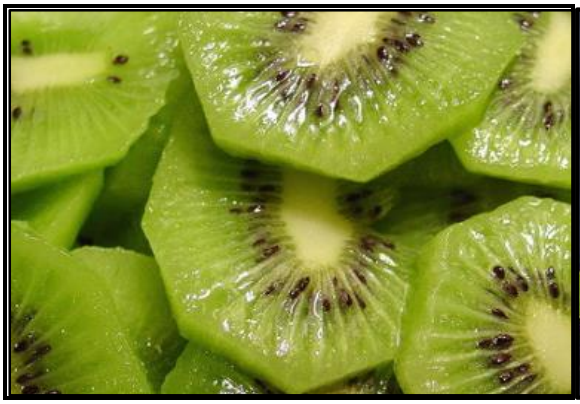


Fig.1 Kiwi fruit

Fig.2 Pineapple fruit

Different chemicals were also Mueller-Hinton agar, HCl, NaOH, sodium citrate buffer , sodium acetate buffer, BSA, Folin Ciocalteau reagent, Actone, alkaline copper sulphate reagent, phosphate buffer, EDTA, L-Tyrosine, casein

Bromelain extraction from pineapple waste

The Mature pineapple pulp, as well as wastes (40 % crown, 40 % peel, and 20 % core), were extracted by grinding in grinder which converted into the liquid form, it was differently with crown, peel, core by which it got easy to stain and separated the liquid for sample. However, kiwi fruit has only peel and pulp, same method was used to get crude original juice.

Source of Microorganisms

The microorganisms used for the study were isolated from Department of Biotechnology, G.H. Raisoni University. The different strain of gram-positive and gram-negative bacteria was present.

Isolation of Microorganisms

1. Culture Preparation:

Preparing agar slants for bacterial isolation in microbiology involves a meticulous process to provide a suitable growth environment for the isolated bacteria. Agar slants are solid media containing agar, a solidifying agent that enables the growth of microorganisms in a vertical position. The preparation of agar slants involves a few key steps and requires specific chemicals and equipment:

1. Materials and Equipment:

• Agar powder

• Distilled water

• Broth medium (e.g., Nutrient Broth)

• Test tubes with caps

• Autoclave or pressure cooker

• Bunsen burner

•Inoculating loop

2. Preparation:

Measure the proper amount of agar powder and distilled water according to the manufacturer's instructions. Mix the agar powder and distilled water in a flask or beaker. Heat the liquid until the agar is completely dissolve. Add the chosen broth medium to the agar solution to provide nutritional support for the bacteria.

3. Sterilization:

To eliminate any remaining germs, autoclave the agar-broth combination at 121°C for 15 minutes. After sterilization, let the agar cool to around 45-50°C before putting it into the test tubes.

4. Pouring the Agar Slants:

Tilt the sterilized and cooled test tubes at an angle (approximately 45°). Carefully pour the agar-broth mixture into the tubes, creating a slanted surface. Allow the agar to harden by cooling the tubes in an upright position.

5. Storage:

To avoid contamination, store the produced agar slants in the refrigerator at 4°C before use. Once prepared, agar slants provide a solid medium for the isolation and cultivation of bacteria. Before use, the agar slants was labelled with the date of preparation, the medium used, and any other relevant information. During bacterial isolation, the agar slants can be inoculated with a sample containing the bacteria of interest using an inoculating loop under aseptic conditions.

Fig.3 Agar slant preparation

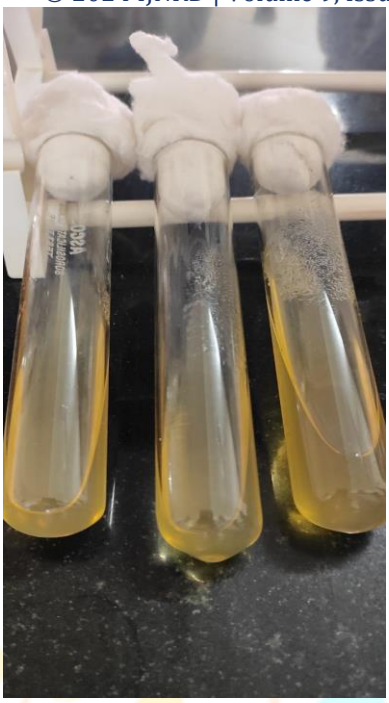


Fig.4 Solidified agar slant for inoculation

6. Inoculate an agar slant from a microbial liquid broth

Prepare workspace by sanitizing plate form and all equipment like pre-sterilized testube, culture tubes, and glassware and needles, including the inoculation loop. To guarantee sterility, heat the inoculation loop until red hot. Remove the broth culture cover and burn the tube's lip. Gently put the loop into the broth culture to extract a tiny amount of liquid. Carefully streak the loop back and forth across the surface of the slant agar to ensure uniform dispersion of the bacterial culture. To avoid contamination, securely replace the cap on the broth culture tube. Incubate the infected agar slant at the proper temperature and duration for bacterial growth. Then test-tube is incubated under 37 degrees Celsius, which is the optimal temperature for the growth of most bacterial cultures for 24 hours. After 24 hours remove from the incubator and sift to refrigerator.

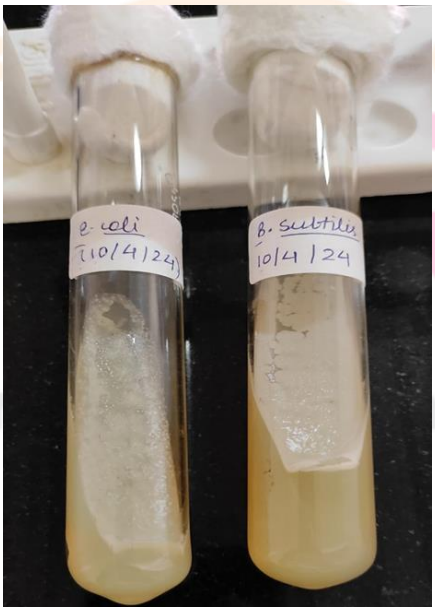


Fig.4 Bacterial culture in agar slant

Preparation of Culture Media

1. Select an appropriate agar medium

The decision to use an appropriate cultural media is critical to the test's effectiveness. Mueller-Hinton agar is recommended because of its low nutrient content and reliable antibacterial susceptibility testing results. The medium's pH should be adjusted to 7.2-7.4 to simulate physiological circumstances and improve bacterial growth. Measure the proper amount of agar powder and distilled water according to the manufacturer's instructions. Mix the agar powder and distilled water in a flask or beaker. Heat is required until the agar is completely dissolved. Add the chosen broth medium to the agar solution to provide nutritional support for the bacteria.

2. Sterilize the media

All equipment like the conical flask in which agar is dissolved, Petri Dishes Etc. should be Wrapped with paper and then arranged into the autoclave. Autoclaves are typically used for heat sterilization, with steam heated to 121-134 °C (250-273 °F) and a holding duration of at least 15 minutes at 121 °C or 3 minutes at 134 °C, longer for liquids and surgical instruments wrapped in layers of fabric.

3. Adjust the pH

Check and adjust the pH of the agar medium to the optimal range for the growth of the test bacteria. Most bacteria prefer a slightly acidic pH range of around 6.8 to 7.2.

4. Pour the agar plates

Light a Bunsen burner and set it to a hot, roaring flame. Collect a bottle of sterile molten nutrient agar from the water bath. Hold the cap of the bottle firmly between the little finger of your left hand and the palm of your left hand. Open the bottle with your right hand by turning. Leave the cap gripped by the little finger of the left hand.Flame the neck of the bottle. Partially open the lid of the Petri dish slightly with your left hand and pour sterile molten agar into the Petri dish. If pouring a series of plates, flame the neck again and again of the bottle again and replace the cap if pausing to avoid contamination. If pouring a single plate from a measured amount into a McCartney/ Universal bottle, place the empty bottle directly in a basin of hot water to make it easier to wash up. Rotate the dish to ensure or conform that the medium covers the base of the dish evenly and properly. The Petri dish base of the plate must be covered, the agar must not touch the lid of the Petri plate, and the surface must be smooth with no bubbles should be appear. If there are bubbles, we can flame the agar surface very briefly to disperse them – but this can carry the risk of melting the Petri dish.

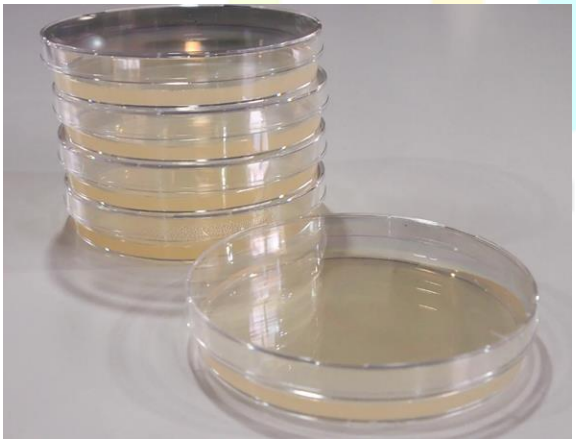


Fig.5 Agar plating for bacterial testing

7. Inoculate the agar plates

Sterilize work surfaces or plate form and equipment as well as prevent contact of sterile instruments and solutions with non-sterile surfaces to avoid contamination. We used the Streak-plating method, which can be accomplished with several different instruments; A metal loop can be re-used multiple times and is utilized for streak-plating routine laboratory strains. Disposable plastic loop I is also available commercially and is used more commonly when working with BSL-2 strains in a Biosafety cabinet. The sample from which the streak plate will be inoculated could be either a suspension of cells in broth or a gram-positive and gram-negative bacterial colony from agar slant.

Take the loop and heat it until the loop becomes red hot with the help of a Bunsen burner. Put the loop to cool down then with the help of loop take the colony from the agar slant this should be performed in front of the Bunsen burner. Open the Petri dish which has solidify touch it lightly on the agar plate, To ensure the loop is not too hot and start the streak-plate procedure spread the colonies on agar. Use a sterile cork borer or pipette tip to create wells in the agar. Add the extract to the well. Incubate the plate at the appropriate temperature for the microorganism.

8. Sample Extraction from kiwi & pineapple & sampling

The fruits are washed with distilled water, peeled and grind into the grinder. To remove the pulp stain it with the help of Muslin cloth (a cotton fabric of plain weave). The liquid extract was separated, which was used as a sample as to natural antimicrobial nature of the fruit. This extracted juice with the help of a micropipette filled into the well (10 µg/ml). An incubator is used to incubate it at 35 C to 36.5 C; use CO2 incubator.

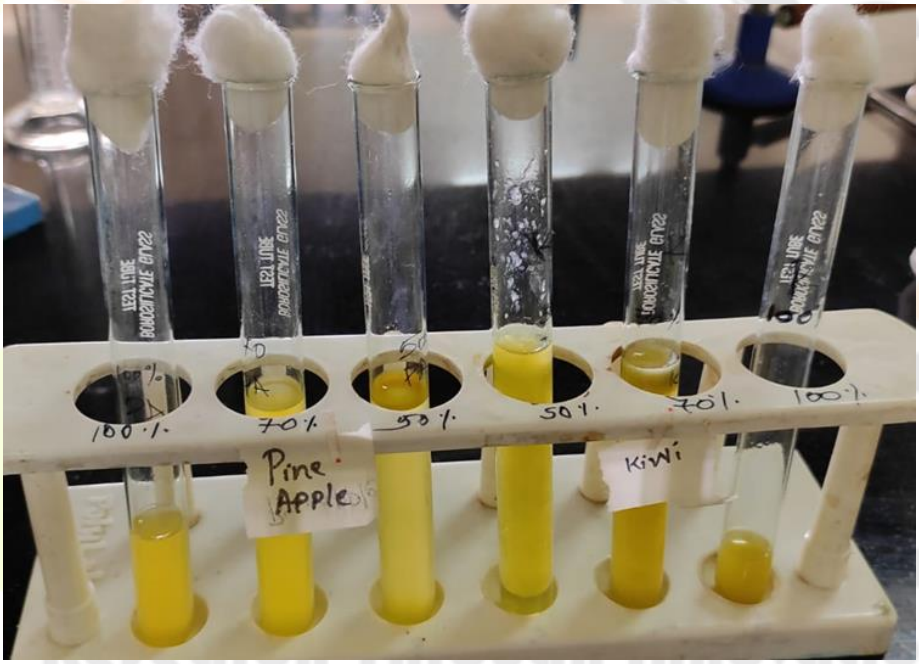


Fig.6 Sample dilution for well diffusion method

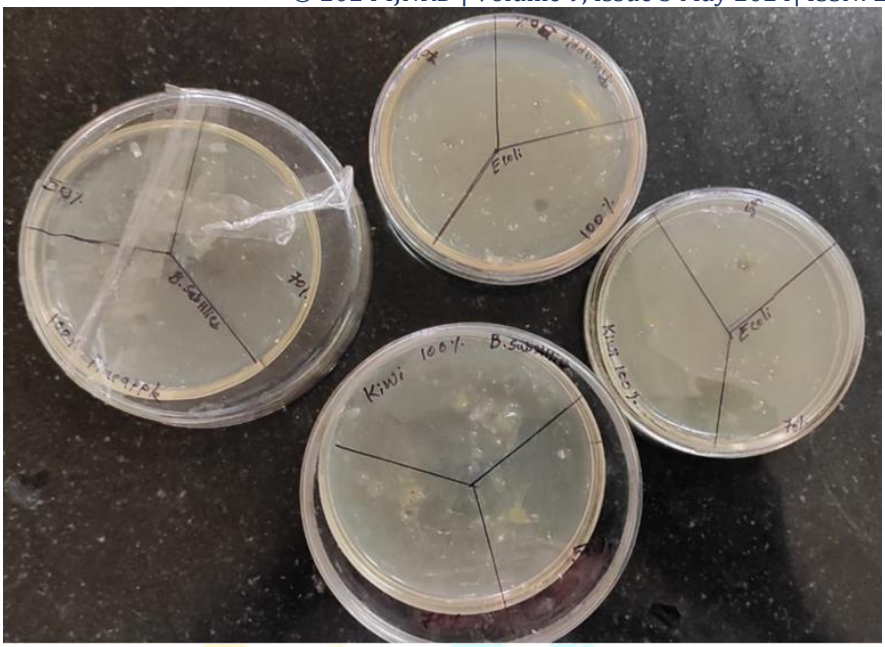


Fig.7 Anti-bacterial activity testing by well diffusion method

IV. EXPERIMENTAL RESULTS

Anti-microbial activity by Well diffusion method:

In this Anti-bacterial activity by Well diffusion method, there are two types of different strains of bacteria are used "Bacillus subtilis" as gram-positive bacteria &" Escherichia coli " as gram-negative bacteria. The plates were incubated at 37ºC for 24 hours.

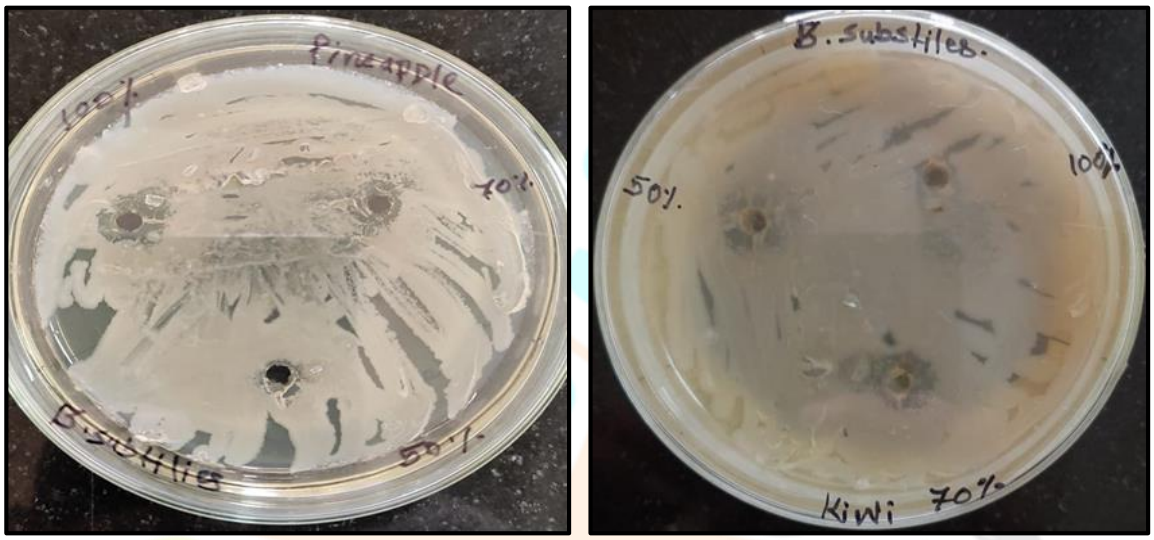
The zone was observed in both "Bacillus subtilis" as gram-positive bacteria &" Escherichia coli " as gram-negative bacteria.The light zone was observed in gram-negative bacteria "Escherichia coli" as compared to gram-positive "Bacillus subtilis" in which small but clear zones was formed & observed in both extracted samples of fruit (Pineapple & Kiwi).

This was performed at different concentration levels like 100%, 70%, 50%. In all sample zone were observed, the diameter of the zone decreased gradually with decrease in concentration as the enzymatic concentration decreased after the dilution of the extracted sample.

Table.1 Show the anti- microbial zone diameter

Extracted sample (%)	Kiwi			Pineapple		
	100%	70%	50%	100%	70%	50%
Organisms	Anti-bacterial Zone Diameter					
Escherichia coli(-)	1.4cm	1.3cm	1.0cm	2.3cm	1.3cm	1.0cm
Bacillus subtilis(+)	1.9cm	1.5cm	1.1cm	1.2cm	0.9cm	0.6cm

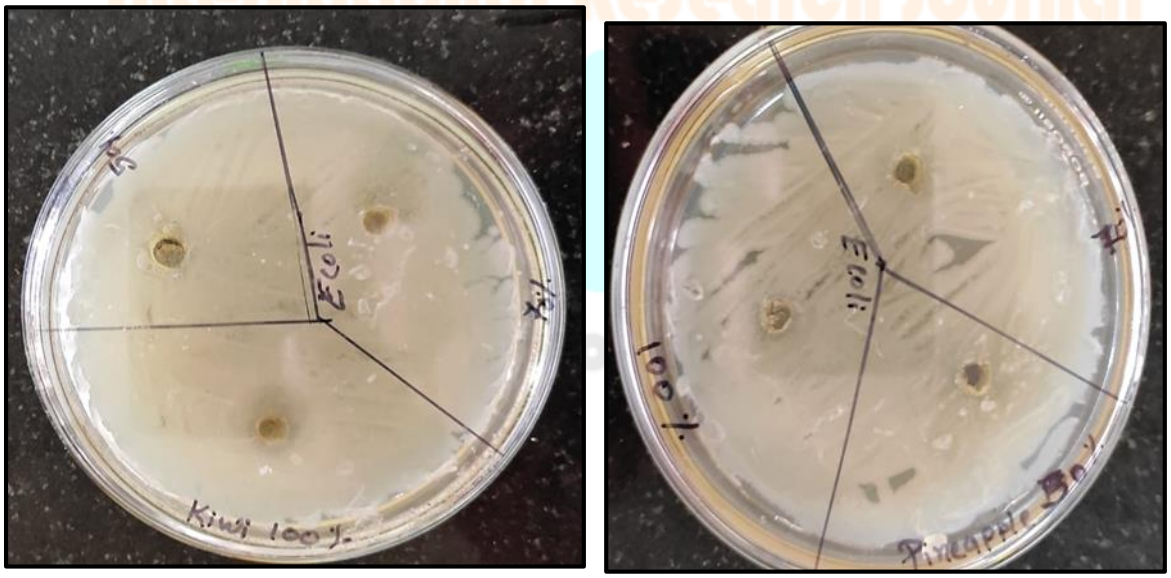
Fig.14 Anti-microbial activity by Well diffusion method



Pineapple Sample **Kiwi sample**

Fig.15 Anti-microbial activity by Well diffusion method

Gram Positive bacteria



Kiwi sample **Pineapple Sample**

Fig.16 Anti-microbial activity by Well diffusion method

Gram Negative bacteria

V. CONCLUSION

The objective of the present study is to perform extraction and separation of Bromelain enzyme from the pineapple fruit material and finding out optimum conditions such as enzyme activity assay for crude extract of Bromelain & Quercetin, effect of pH on enzyme activity, effect of preservatives on enzyme activity, Kinetic studies on activity, stability of the crude Bromelain & Quercetin extract was performed successfully. The pH of the extract is in the range from 3 to 4 for both species.

Bromelain *omelain* is a plant cysteine protease enzyme extracted from the stem and leaf of pine apple (*Ananas comosus*). The bromelain extracted from the peel has high bromelain activity of 246.83 CDU/ml (without crown) and 228.9 CDU/ml (with crown); only slightly lower than the flesh. Thus results revealed that pineapple waste such as peels which contribute to almost 30% of pineapple waste can be a good source of bromelain. Because of its proteolytic nature, kiwifruit enzyme has many applications in food, including replacing rennet in cheese production, reducing fermentation time in bun production, and tenderizing spent hen chicken, in addition to traditional plant proteases such as papain, quercetin, ficin, and bromelain.

It has been shown to have an effective antibacterial impact on both gram negative and gram positive bacteria, including Escherichia coli and Bacillus subtilis. The flesh of the pineapple is the greatest component (w/w) for both crown and crownless pineapples, followed by the peel, core, stem, and crown. The flesh and peel exhibit the highest bromelain activity. However, peel is the major waste proportion and seems to have the utmost potential for bromelain extraction.

A specific strain of E. coli and Bacillus subtilis were resistant to crude bromelain. The crude extract was most potent at 25°C - 37°C in normal pH medium against E. coli and Bacillus subtilis respectively. The crude enzyme showed better activity against Bacillus subtilis at pH 9.0. Combination of the crude bromelain and an antibiotic had most effect than either standard or crude bromelain.

The higher temperature and pH stability of kiwifruit enzyme show its potential for industrial applications. The polyphenol extracted from kiwi fruit seeds was found to possess promising antimicrobial activities. The effectiveness of bromelain as a anti-browning agent which can commercially available anti-browning agents for comparison.

This give the positive feedback to the experiment as the main motive this experiment was to identified comparative study of different enzyme present in fruit to get natural effect against different strain of bacterial.

This can be further use for the human welfare against the microbal infection & diseases.

REFERENCES

[1] Holyavka M, Faizullin D, Koroleva V, et al.: Novel biotechnological formulations of cysteine proteases, immobilized on chitosan. Structure, stability and activity. Int J Biol Macromol. 2021, 180:161-76. 10.1016/j.ijbiomac.2021.03.016

[2] Smith-Marshall J. Golden KD. Characterization of Bromelain from Morinda citrifolia (Noni). J Sci Res. 2012; 4(2): 445-456.

[3] Ataide JA, Cefali LC, Figueiredo MC, et al.: In vitro performance of free and encapsulated bromelain . Sci Rep. 2021, 11:10195

[4] Smith-Marshall J. Golden KD. Characterization of Bromelain from Morinda citrifolia (Noni). J Sci Res. 2012; 4(2): 445-456.

[5] Tochi BN, Wang Z, Xu SY, Zhang W. Therapeutic Application of Pineapple Protease (Bromelain): A Review. Pakistan J Nutrition. 2008; 7 (4): 513-520.

[6] Upadhyay A, Lama JP, Tawata S. Utilization of Pineapple Waste: A Review. J Food Sci Technol. 2010; 6(1): 1018

[7] Neta JLV, DaSilva LA, Lima AA, Santana JC, Leite NS, Ruzene DS, Silva DP,De Souza RR. Bromelain Enzyme from Pineapple: In Vitro Activity Study under Different Micropropagation Conditions. Appl Biochem Biotechnol. 2012; 168(2): 234- 246.

[8] Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C., Angioni, A., Dessiet, S., et al. (2008). Comparative analysis of polyphenolic profiles and antioxidant and antimicrobial activities of Tunisian pome fruit pulp and peel aqueous acetone extracts. J Agri Food Chem 56:1084– 1090.