



Contents lists available at ScienceDirect

## Saudi Journal of Biological Sciences

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Original article

## Applications of bromelain from pineapple waste towards acne

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## ARTICLE INFO

## Article history:

Received 23 July 2020

Revised 26 October 2020

Accepted 4 November 2020

Available online 11 November 2020

## Keywords:

Bromelain

Acne

Antioxidant

Antimicrobial

Formulation

## ABSTRACT

Bromelain is a proteolytic mixture obtained from pineapple (*Ananas comosus* (L. Merr)). It has diversified clinical properties and is used in alleviation of cancer, inflammation and oxidative stress. The current study focuses on extraction of bromelain from different parts of pineapple such as core, crown, fruit, peel and stem. The extracted enzyme was precipitated using ammonium sulphate at 40% saturation followed by dialysis. The fold of purification obtained for peel, crown, core, fruit and stem were found to be 1.948, 1.536, 1.027, 1.989, and 1.232 respectively. Bromelain activity was estimated using Azocasein assay, the highest activity was seen in peel at 3.417 U/ $\mu$ g. Antimicrobial activity and MIC of the bromelain purified and crude fractions was studied against the test organisms. Peel crude and purified extract exhibited highest inhibitory effect towards *S. aureus* followed by *P. acne*. The antioxidant activity was evaluated using DPPH antioxidant assay. IC50 values peel, fruit, stem and crown are found to be 13.158  $\mu$ g/ml, 24.13  $\mu$ g/ml and 23.33  $\mu$ g/ml and 113.79  $\mu$ g/ml respectively. The purified bromelain from peel, stem and crown was used to create a facewash formulation towards pathogens frequently associated with skin infections. Common skin pathogens like *S. aureus* and *P. acne* were found highly sensitive to its action. The aim of this study was to evaluate the potential of bromelain isolated from waste parts of pineapple in alleviation of acne due to its diverse antimicrobial properties.

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## 1. Introduction

Pineapple (*Ananas comosus*) has been used as a traditional medicine by several cultures throughout time and bromelain has been established since 1876. Bromelain gets its properties mainly due to the presence of its sulphhydryl proteolytic enzymes. Bromelain is classified as stem bromelain or fruit bromelain depending on the origin of the protease. Bromelain is present throughout the pineapple plant however the concentration and composition may vary depending on the part of the fruit and its variety (Gautam et al., 2010). Extraction of bromelain makes it feasible as its bioavailability rises during maturity of fruit rather than during fruit development (Maurer, 2001).

Extensive studies have been done with bromelain to explore its clinical properties. Thus, finding effective extraction methods is necessary. Bromelain is found in pineapple wastes such as core,

peel and leaves in reduced quantities compared to the fruit (Sriwatanapongse et al., 2000). Various purification strategies have been discussed and developed for extraction of bromelain. It precipitates at 20% to 60% with ammonium sulphate showing highest recovery at 20%. Dialysis to be followed after ammonium precipitation to concentrate and to purify bromelain further by a cost-effective manner (Pardhi et al., 2016).

Skin problems like wrinkles, acne and dry skin seemed to be solved using proteases such as papain and bromelain (Ozlen and Chatsworth, 1995). Loon et al., (2018) observed sensitivity of *S. aureus* against pineapple extract. Partially purified bromelain from the pineapple core also inhibited the growth of *S. epidermidis* and *P. acne* (Hidayat et al., 2018). Bromelain has also been extensively used as a line of treatment for periodontal gingivitis and found to be effective against periodontal pathogens namely, *S. mutans*, *A. actinomycetemcomitans*, *P. gingivalis* (Praveen et al., 2014). Edema caused by post-surgical trauma also has been effectively treated by bromelain (Mackay and Miller, 2003).

Acne is a skin condition affecting the skin's oil glands is a very prominent condition. It can create a lot of psychological disturbances and stress on the individual that it affects. *P. acne* is an opportunistic pathogen that plays an important role in the growth and cause of acne. *S. aureus* is a part of the normal skin microbiota and is associated with skin conditions such as folliculitis and also

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Peer review under responsibility of King Saud University.



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reported to enhance the effect of other microbes in acne lesions (Kumar et al., 2016).

Hence this study aims to evaluate the potential of bromelain in alleviation of acne owing to its diverse antimicrobial properties. Active bromelain was isolated from waste parts of pineapple and its effects on bacterial pathogens like acne was studied. Bromelain will aid as a potent anti-inflammatory agent that will act as beneficial additive to the formulation. Using bromelain from the waste parts of pineapple will help in waste recycling as well as make the whole process cost-effective.

## 2. Materials

Biological material: Pineapple, Turmeric, Aloe vera gel

Chemicals: Acetic acid (Chemdyes corp.), Agar Agar Type 1 (Hi-Media), Ammonium Sulphate (Loba Chemie), Ampicillin antibiotic discs (Hi-Media), Ascorbic Acid (Loba Chemi), Azocasein (Sigma-Aldrich), Beef Extract (Hi-Media), Bovine Serum Albumin (Sigma-Aldrich), Conc. HCl (Chemdyes), Copper Sulphate, DPPH (Hi-Media), Folin-Ciocalteu reagent (ThermoFisher scientific), Peptone (Hi-Media), Sodium Acetate Trihydrate, Sodium Carbonate, Sodium Chloride (SDFCL chemicals), Sodium Hydroxide, Sodium Potassium Tartrate, Tris Base (Hi-Media), Tris HCl (Hi-Media), Yeast Extract (Hi-Media)

Microbiological Strains: *S. aureus* (MTCC 96), *C. diphtheriae*, *E. coli* (ATCC: 25922), *Pseudomonas aeruginosa* (ATCC: 227853), *P. acne* (MTCC: 1951).

## 3. Methodology

### 3.1. Sample preparation

#### 3.1.1. Crude extract preparation

The pineapple parts were categorized as fruit, stem, peel, core and crown. They were individually homogenized with 0.1 M of sodium acetate buffer, (pH – 7.0). The extracts obtained was filtered and centrifuged at 6000 rpm for 20 min at 4 °C. These extracts were labelled as fruit crude extract, stem crude extract, peel crude extract, core crude extract and crown crude extract of bromelain. The parts are observed in Fig. 1.

#### 3.1.2. Ammonium sulphate precipitation

The crude extract was subjected to ammonium sulphate precipitation at 40% saturation (226 g/l). The extraction was carried out in an ice box over a magnetic stirrer. Ammonium sulphate was gradually added pinch by pinch in a period of 30 min. It was allowed to settle for 24 h at 4 °C. The solution was then centrifuged at 6000 rpm for 10 min at 4 °C. The pellet was later reconstituted in minimum volume of 10 mM Tris, (pH – 7.0) and then subjected to dialysis. The supernatant obtained was further precipitation at 60% saturation (120g/l). The solution was allowed to stand for 24 h at 4 °C and then centrifuged at 6000 rpm for 10 min at 4 °C. The pellet obtained was reconstituted in minimum volume of 10 mM Tris, (pH – 7.0).

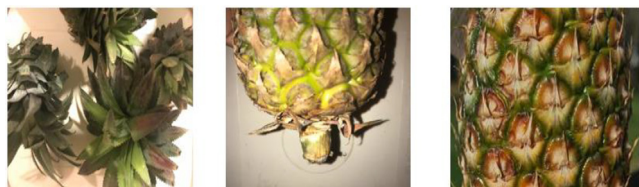


Fig. 1. Parts of the pineapple, Crown, stem, peel (left to right).

### 3.1.3. Dialysis

**3.1.3.1 Activation of dialysis membrane.** 7 cm of dialysis membrane was added into boiling distilled water with 2% sodium carbonate for 45 min. The membrane was boiled again for 45 min in distilled water, the membrane was left overnight in Acetate buffer (pH-7.0).

**3.1.3.2 Dialysis.** The fractions from ammonium precipitation were loaded into the activated dialysis membranes and tagged. They were equilibrated into a beaker with acetate buffer. The process was carried out for 24 h in an ice box with the replacement of buffer every 6 h. The samples from membranes were then unloaded and labelled as purified bromelain samples. The setup is observed in Fig. 2.

### 3.2. Estimation of protein content by Folin-Lowry assay

The protease mixtures were estimated for the protein content by using 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% Na<sub>2</sub>CO<sub>3</sub> in 0.1 N NaOH: 0.5% CuSO<sub>4</sub> 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 ( $\Lambda_m = 660$  nm). (Plummer and Plummer, 1988)

### 3.3. Estimation of proteolytic activity

#### 3.3.1. Calibration curve

Azocasein was used as the substrate in the concentration range of 0.1–2%. Azocasein was digested using enzyme sample for 300 min and using regression analysis, an equation was derived correlating the absorbance/color intensity to the substrate concentration.

#### 3.3.2. Proteolytic activity of samples

Proteolytic activity of samples was tested varying the azocasein concentration from 0.1 to 2%. Equal quantities of substrate and enzymatic samples (i.e. 100  $\mu$ l) were incubated for 10 min. The reaction was terminated using 5% Trichloroacetic acid (TCA) and the vials are centrifuged at 4 °C for 10 min. The supernatant is taken by 1:1 dilution of 0.5 N NaOH and its absorbance is read ( $\Lambda_m = 440$  nm). The blank was obtained by mixing the TCA to the substrate prior to enzyme addition. Calculations were done as specified by Coelho et al. (2016)

### 3.4. Antimicrobial activity testing

#### 3.4.1. Bacterial maintenance

*S. aureus* (MTCC 96), *C. diphtheria*, *E. coli* (ATCC: 25922), *P. aeruginosa* (ATCC: 227853), *P. acne* (MTCC: 1951) were maintained on Nutrient Agar slants and incubated at 37 °C. These cultures are going to be further mentioned as test cultures.



Fig. 2. Dialysis setup, Sample loaded in dialysis membrane (Left to right).

### 3.4.2. Ditch plate technique

Antimicrobial susceptibility of the organisms towards the crude extract was observed by using the ditch plate technique. Bromelain extract was mixed with molten agar and poured into the ditch. The test organism is plated across the ditch. The plates incubated at 37 °C for 24 h. The Zone of Inhibition was measured (Benson et al., 2015).

### 3.4.3. Minimum inhibitory concentration by turbidity test

The MIC of the purified extracts of peel, fruit and stem against the test cultures in a 10–100% (v/v) dilution. Saline was used as a negative control in place of test samples. Absence of turbidity signifies no growth of culture whereas presence of turbidity signifies growth of culture (Wiegand et al., 2008).

### 3.5. Antioxidant activity testing by DPPH method

Antioxidant activity (DPPH free radical scavenging activity): The antioxidant activity of the extracts and standards was based on the radical scavenging effects of 2, 2 diphenyl-1-picrylhydrazyl (DPPH). The diluted working solutions of the samples were made in water. Ascorbic acid was used 10–100 µg/ml. 0.002% DPPH was prepared in ethanol. The test sample/standard was mixed with DPPH in 1:1 ratio and incubated in the dark for 30 min. The solution mixtures were read at 517 nm. The OD was recorded and percentage inhibition was calculated by the formula below. Ethanol with DPPH (1:1) is used as a blank (Khalaf et al., 2008; Yuris and Siow, 2014).

$$\text{Percent inhibition} = \left( \frac{A - B}{A} \times 100 \right)$$

where A is OD of blank and B is OD of sample.

### 3.6. Formulation

Three formulations were synthesized using the stem bromelain, peel bromelain and crown bromelain as the primary base See Table 1 and Fig. 3.

**Table 1**  
Formulation of Bromelain Facewash.

Material	Function
Bromelain	Antimicrobial/Antioxidant
Turmeric	Antioxidant
Aloe Vera Gel	Moisturizer
Xanthum gum	Thickener
Tea tree oil	Essential oil/Preservative
NaOH, SLES, Sodium stearate, Propylene glycol	Soap Base



**Fig. 3.** Bromelain Facewash Formulations.

### 3.6.1. Physical characteristics of the facewash

Physical characteristics were studied such as the color, pH, washability, foamability and consistency (Ingle and Meshram, 2018).

### 3.6.2. Antibacterial testing of formulation

The antimicrobial activity of formulation the crude extract was determined using the disc diffusion method. Nutrient agar plates were swabbed with the cultures. Sterile Whatmann discs impregnated with the test samples were placed. The samples were tested in four concentrations of (v/v) percentage at 25%, 50%, 75% and 100%. They were incubated at 37 °C for 24 h. The Zone of Inhibition was measured. (Benson et al., 2015)

### 3.7. Comparison of commercial products

Four commercially available herbal facewashes were selected at random and their antimicrobial action against bacterial cultures were tested at four (v/v) % concentration levels (i.e. 25%, 50%, 75%, 100%). These were named as Brand 1, Brand 2, Brand 3 and Brand 4)

## 4. Results and conclusion

### 4.1. Estimation of protein content

The protein content was estimated by folin-lowry method. The values obtained in crude extracts were fruit (170 µg/ml), crown (167 µg/ml), peel (128 µg/ml), stem (127 µg/ml) and core (126 µg/ml). The crude extracts were purified by ammonium sulphate precipitation and dialysis. The highest protein content after purification was observed in peel (103 µg/ml), followed by stem (100 µg/ml), crown (93 µg/ml) and core (92 µg/ml). The results are shown in Graph 1.

### 4.2. Estimation of proteolytic activity

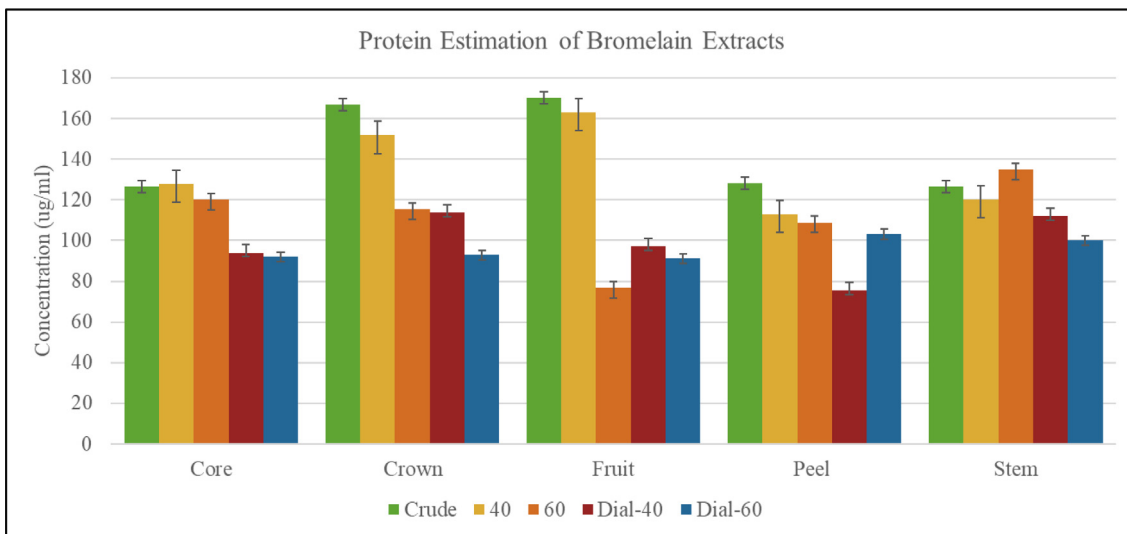
Proteolytic activity was estimated using the Azocasein method. Azocasein content was calibrated for maximum digestion using bromelain as seen in Graph 2. This was used for calculation of proteolytic activities of crude and purified extracts. An increase was seen in proteolytic activities after purification. Peel and fruit demonstrated highest proteolytic activity at 3.417 U/µg and 2.556 U/µg. Proteolytic activities have been depicted in Graph 3 and Table 2.

### 4.3. Estimation of antimicrobial susceptibility testing of bromelain extracts

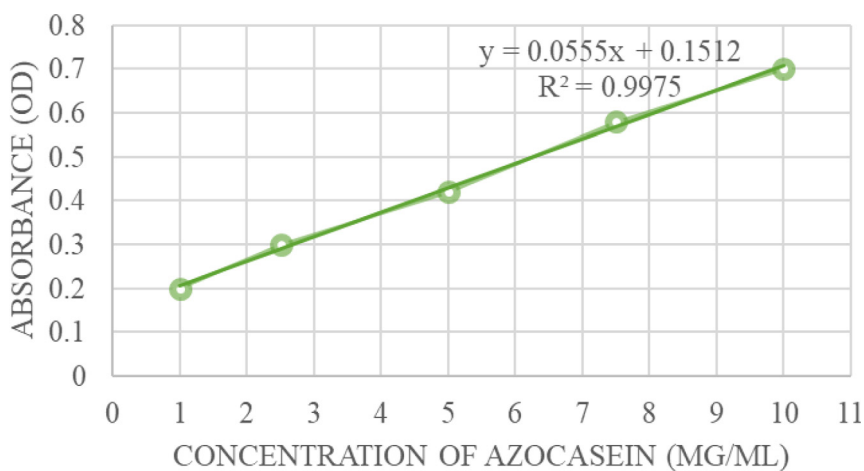
Antimicrobial susceptibility testing of crude and purified bromelain extracts was performed by ditch plate method. Peel crude and purified extract exhibited highest inhibitory effect towards *S. aureus* followed by *P. acne*, *E. coli*, *C. diphtheria* and *P. aeruginosa*. *P. aeruginosa* was most susceptible to the action of bromelain. According to inhibitory action, the potent extracts were peel, fruit, stem, crown and core. The results are shown in Graphs 4, 5 and Fig. 4.

### 4.4. Estimation of minimum inhibitory concentration (MIC) by turbidity test

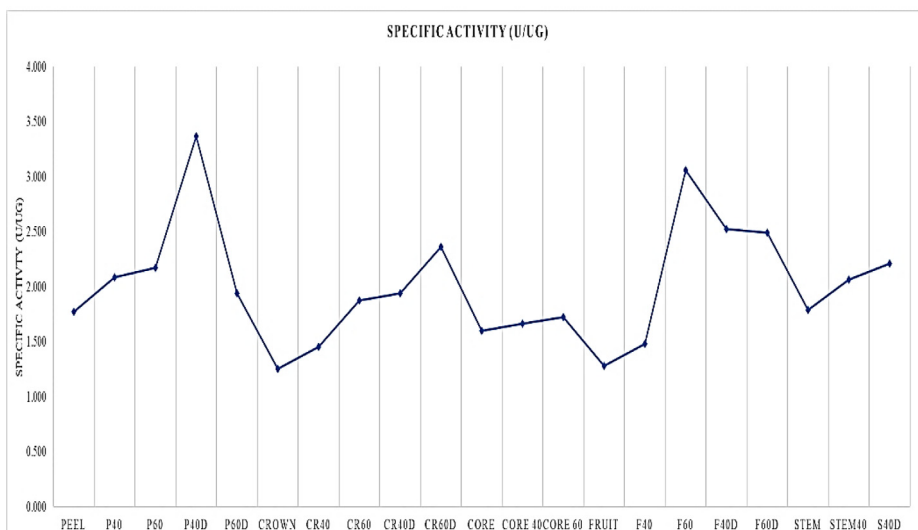
Minimum Inhibitory concentration of the purified extracts was studied against the test cultures. *P. acne* was found susceptible against the purified fractionates at concentrations as low as 19 µg/ml of the fruit extract. While *P. aeruginosa* and *C. diphtheria*



Graph 1. Estimation of protein content in bromelain crude and purified extracts.



Graph 2. Calibration Graph of Azocasein.

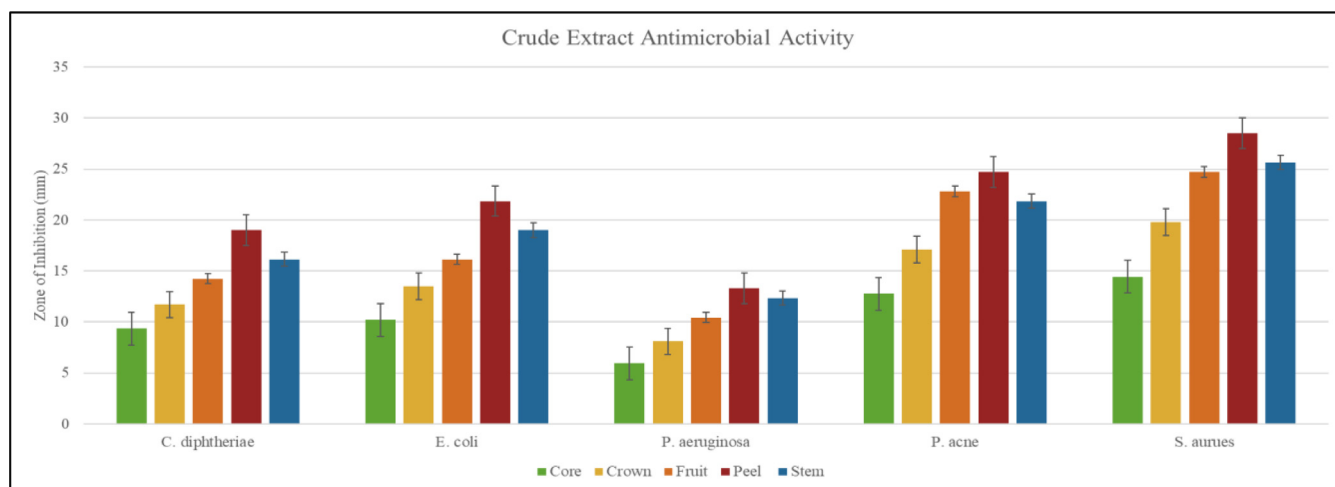


**KEY**  
 P – Peel  
 Cr – Crown  
 C – Core  
 F – Fruit  
 S – Stem  
  
 40 – 40% amm. Sulp saturation  
 60 – 60% amm. Sulp. Saturation  
  
 40D – 40% saturation – subjected to dialysis  
 60D – 60% saturation – subjected to dialysis

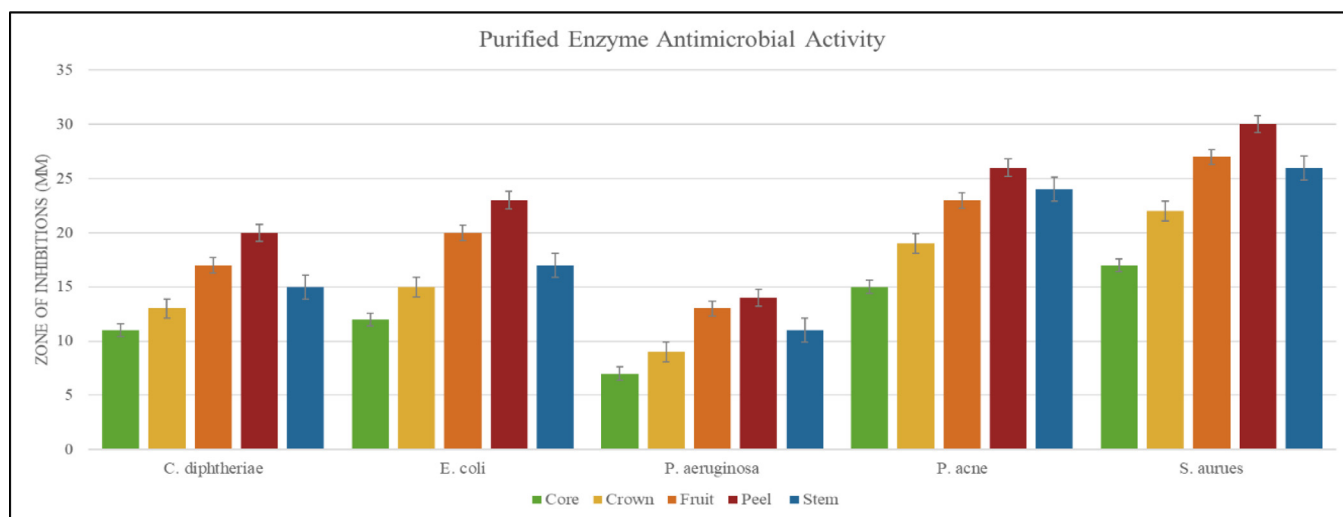
Graph 3. Specific activity exhibited by Bromelain fractions by Azocasein assay.

**Table 2**  
Azocasein assay summation.

	Specific Activity of Crude (U/ug)	Specific Activity of Purified fraction (U/ug)	Fold purification	Percentage Yield (%)
Peel	1.755	3.417	1.948	112.519
Crown	1.258	1.933	1.536	105.453
Core	1.615	1.658	1.027	102.435
Fruit	1.285	2.556	1.989	114.032
Stem	1.798	2.216	1.232	108.627



**Graph 4.** Antimicrobial activity exhibited by Crude extracts of Bromelain.



**Graph 5.** Antimicrobial activity exhibited by purified Bromelain.

are susceptible, they require higher concentration of the enzyme to inhibit its growth. Turbidity test observations are shown in Table 4. MIC values are shown in Graph 6 and Table 34.

**4.5. Antioxidant activity**

Natural antioxidants are present in bromelain that help in combating oxidative stress. The antioxidant activities of various bromelain samples are found to significantly different. (One-Way ANOVA;  $\alpha$ -0.05; P value – 0.703). IC50 values bromelain samples

of peel, fruit, stem and crown are 13.158  $\mu$ g/ml, 24.13  $\mu$ g/ml and 23.33  $\mu$ g/ml and 113.79  $\mu$ g/ml. The antioxidant activity of bromelain is compared with ascorbic acid (used as a standard). IC50 of ascorbic acid being 9.92  $\mu$ g/ml. The percentage inhibition of bromelain samples are shown in Graph 7 and Table 5.

**4.6. Physical parameter testing of test formulations**

The physical parameters of the test formulations have been depicted in Table 6.





Fig. 4. A petriplate showing antimicrobial sensitivity by Bromelain by Ditch plate technique.

4.7. Antimicrobial susceptibility results of Bromelain test formulation

The antimicrobial susceptibility of the formulation was tested by disc diffusion against the test organisms. *S. aureus* was the most

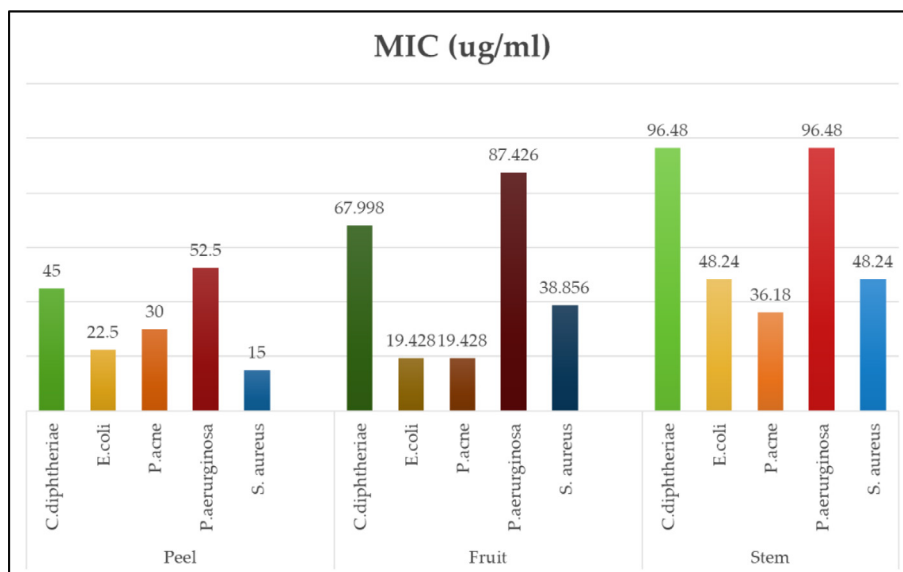
susceptible organism by the three test formulations followed by *P. acne*. The zone of inhibition is graphically represented. Results are depicted in Graph 8 and Fig. 5.

4.8. Antimicrobial susceptibility results of commercial products

All the facewashes were found to be effective against *P. acne* and *S. aureus*. However, Brand 2 and brand 3 showed no inhibition against *E. coli*. Results are shown in Graph 9 and Fig. 6.

5. Discussion

Pineapple is an age-old fruit, consumed and used traditionally for many of its medicinal properties. It has been used for debridement of skin (Houck et al., 1983). Bromelain is also used as a replacement during deficiency of pepsin and trypsin due to its activity and stability over a wide pH range (Balakrishnan et al., 1981). Generally, only the fruit is considered as the only edible member and the rest is treated as waste i.e. the peel, the core, crown and the stem. Pineapple wastes are usually discarded and then allowed to biodegrade which leads to environmental degradation mainly due to carbohydrate rich contents. According to our



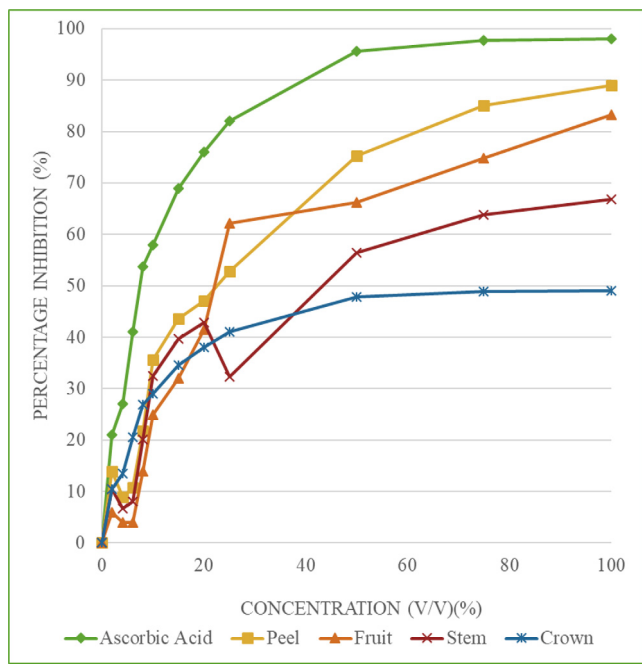
Graph 6. MIC results of extracts against cultures.

Table 3  
MIC results of extracts v/s culture.

Part	Organism	MIC (ug/ml)
Peel	<i>C.diphtheriae</i>	45
	<i>E.coli</i>	22.5
	<i>P.acne</i>	30
	<i>P.aeruginosa</i>	52.5
	<i>S.aureus</i>	15
Fruit	<i>C.diphtheriae</i>	67.998
	<i>E.coli</i>	19.428
	<i>P.acne</i>	19.428
	<i>P.aeruginosa</i>	87.426
	<i>S.aureus</i>	38.856
Stem	<i>C.diphtheriae</i>	96.48
	<i>E.coli</i>	48.24
	<i>P.acne</i>	36.18
	<i>P.aeruginosa</i>	96.48
	<i>S.aureus</i>	48.24

**Table 4**  
MIC turbidity test observations.

		Peel Purified Extract									
Organism	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	
<i>C. diphtheria</i>	-	-	-	-	-	+	+	+	+	+	
<i>E. coli</i>	-	-	-	-	-	-	-	-	+	+	
<i>P. acne</i>	-	-	-	-	-	-	-	+	+	+	
<i>P. aeruginosa</i>	-	-	-	-	+	+	+	+	+	+	
<i>S. aureus</i>	-	-	-	-	-	-	-	-	+	+	
		Fruit Purified Extract									
Organism	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	
<i>C. diphtheria</i>	-	-	-	-	+	+	+	+	+	+	
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	+	
<i>P. acne</i>	-	-	-	-	-	-	-	-	-	+	
<i>P. aeruginosa</i>	-	-	+	+	+	+	+	+	+	+	
<i>S. aureus</i>	-	-	-	-	-	-	-	+	+	+	
		Stem Purified Extract									
Organism	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	
<i>C. diphtheria</i>	-	-	-	+	+	+	+	+	+	+	
<i>E. coli</i>	-	-	-	-	-	-	-	+	+	+	
<i>P. acne</i>	-	-	-	-	-	-	-	-	+	+	
<i>P. aeruginosa</i>	-	-	-	+	+	+	+	+	+	+	
<i>S. aureus</i>	-	-	-	-	-	-	-	+	+	+	



**Graph 7.** Antioxidant activity of Ascorbic acid and Bromelain extracts.

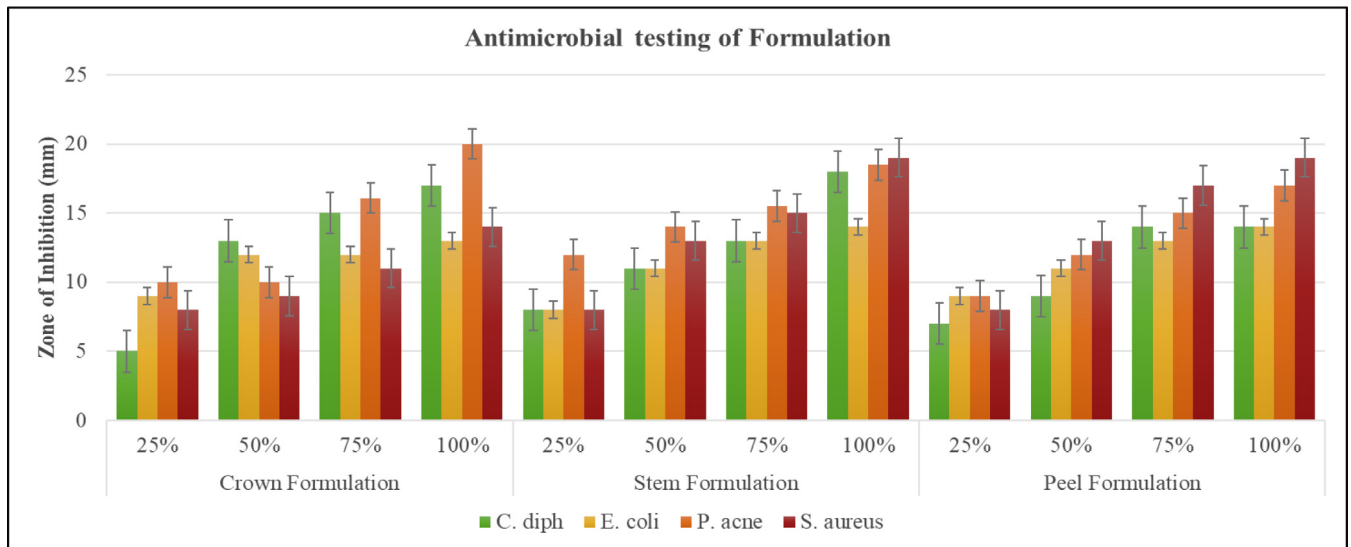
**Table 5**  
IC50 values exhibited by the samples tested.

Sample	IC50 value
Ascorbic Acid	9.92 µg/ml
Peel	13.158 µg/ml
Fruit	24.13 µg/ml
Stem	23.33 µg/ml
Crown	113.79 µg/ml

**Table 6**  
Physical observations of the formulations.

Factor	Peel Formulation	Stem Formulation	Crown Formulation
pH	5.5	5.9	5.3
Color	Golden Yellow	Brownish Yellow	Pale Green
Consistency	Semi-solid	Semi-solid	Semi-solid
Washability	Good	Good	Good
Foamability	Low Foam appears	Low Foam appears	Low Foam appears

study, active bromelain was obtained from the waste parts as well. Peel demonstrated highest proteolytic activity at 3.417 U/µg followed by fruit at 2.556 U/µg. Ammonium sulphate precipitation and dialysis were applied for purification for the ease of usage, feasibility and easy recovery of the enzyme with highest activity. This step is in accordance with (Pardhi et al., 2016) which validate the use of these methods for bromelain purification. Bromelain activity was studied using azocasein assay. The highest activity was seen in peel and fruit at 3.417 U/µg and 2.556 U/µg respectively. This method is in accordance with (Coêlho et al., 2016) for estimation of proteolytic activities using azocasein. Yuris and Siow (2014) associated pineapple for its antioxidant activities to the presence of phenolics, which motivated us to study if bromelain also exhibited antioxidant activities. IC50 values of bromelain samples of peel, fruit, stem and crown are 13.158 µg/ml, 24.13 µg/ml and 23.33 µg/ml and 113.79 µg/ml. Acne is a skin condition affecting the skin's oil glands is a very prominent condition. It can create a lot of psychological disturbances and stress on the individual that it affects. The microbial flora often associated with acne infection includes *P. acnes*, *S. epidermidis*, and *S. aureus*. The antimicrobial activity of bromelain was tested against *P. acne*, *S. aureus*, *C. diphtheria*, *E. coli* and *P. aeruginosa*. *S. aureus* was the most susceptible organism to the action of crude and purified bromelain extracts followed by *P. acne*. Peel crude and purified extract exhibited highest inhibitory effect towards *S. aureus*. The highest resistance to Bromelain activity was shown by *P. aeruginosa*. A facewash was prepared using peel, stem and crown bromelain extracts. To the best of our knowledge, this is the first formulation that was prepared using bromelain extracted from the wastes of pineapple. The antimicrobial susceptibility of formulation was tested by disc diffusion against the test organisms. *S. aureus* was the most susceptible organism by the three test formulations followed by *P. acne*. We chose four popular herbal facewashes acclaimed to be used for anti-acne and compared the antimicrobial action of the formulations. Brand 2 and Brand 3 showed no activity against *E.coli*. The



Graph 8. Antimicrobial testing of Bromelain facewash formulations.

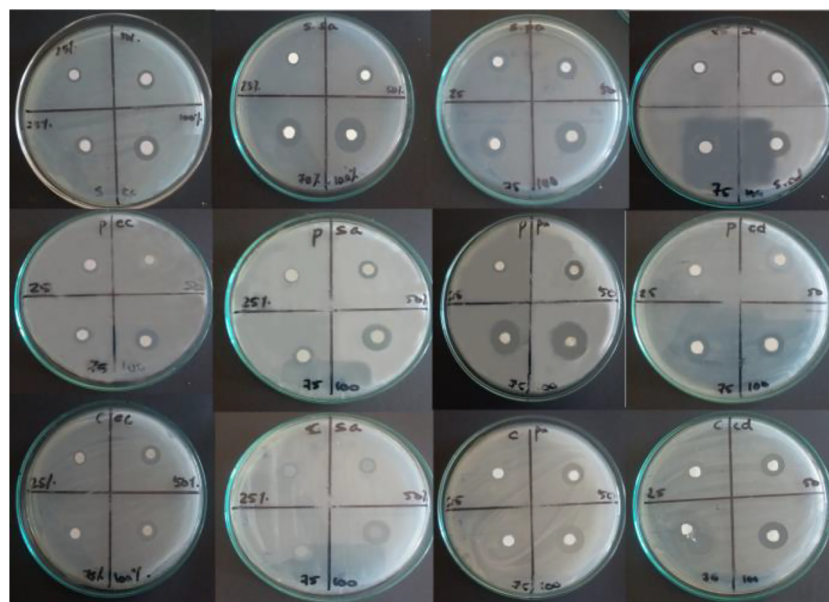
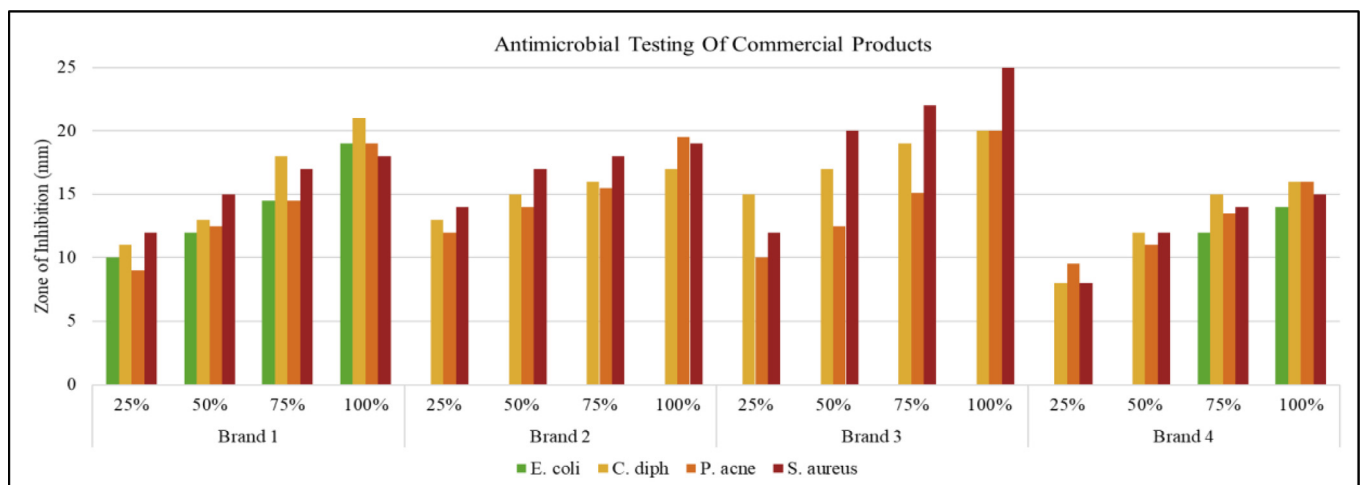


Fig. 5. AST of Bromelain Formulation: stem, peel and crown (Top to bottom) against E. coli, S. aureus, P. acne, and C. diphtheria (left to right).



Graph 9. Antimicrobial testing of commercial facewashes.



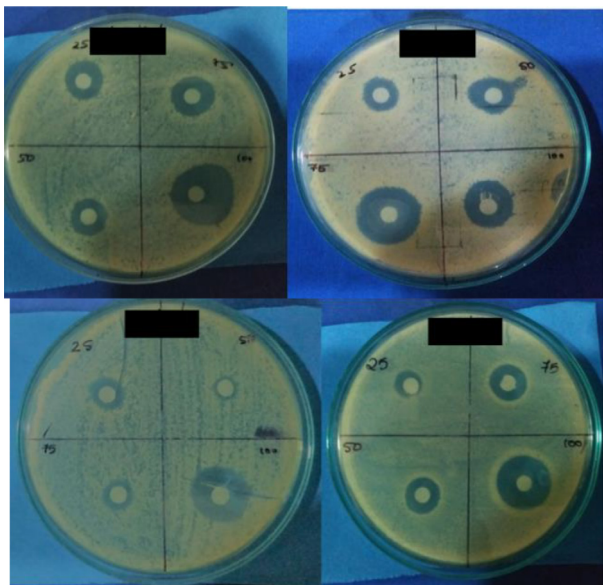


Fig. 6. AST of commercial formulations against *P. acne*.

bromelain formulations showed activity against *P. acne*, *S. aureus*, *C. diphtheria* and *E. coli*. Comparison of bromelain and commercial facewashes found a difference in activity against *S. aureus* and *P. acne*.

## 6. Conclusions

Bromelain has proved to be a potential protease that can be used clinically for treatment of acne owing to its antimicrobial activity, ease of purification and extraction from waste parts of the pineapple. It is a good candidate since the purified protease is potent and the extraction is feasible bromelain also has a wide stability range in terms of temperature and pH. The antioxidant property of bromelain also contributes to the benefit of skin in battling oxidative stress. Acne has always been a cause of great physical and social distress due to its sensitive nature, one has to be very cautious around acne to prevent another outbreak causing more distress to the skin. The bromelain formulation was the first to be made from the wastes of pineapple. Bromelain can also be further explored to study it as a bactericidal gel with direct treatment for acne, however it's interaction with other skin flora may also need to be studied to see if it is a selective protease or not. Bromelain interactions with other skin proteases and antimicrobial peptides such as psoriasin can also be explored.

## Acknowledgments

This project was funded by University of Mumbai minor research grants scheme, URG project – 388. We would like to extend our gratitude to National Facility for Biopharmaceuticals at Guru Nanak Khalsa College for their sophisticated instrumentation and analysis support.

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