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Antimicrobial effects of crude bromelain extracted from pineapple fruit (*Ananas comosus* (*Linn.) Merr.*)

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Abstract: The study assessed the antimicrobial activity of crude bromelain extracted from pineapple fruit (*Ananas comosus L.*) on some microorganisms isolated from fresh and overnight meat at different temperatures and pH. Bromelain was extracted from pineapple fruit by homogenizing in cold phosphate buffer solution. Crude bromelain was estimated by Bradford method and the enzyme was assayed by the casein digestion method. Six bacteria namely, *Proteus spp*, *Corynebacterium spp*, *B. subtilis*, *S. pyogenes* and two different strains of *E. coli*., were isolated and identified by the conventional methods. The antimicrobial activity of crude bromelain was determined by the disc diffusion method. One strain of *E. coli* had the highest zone of inhibition (24.00±1.53mm) at 25°C, but the other strain was resistant. *Corynebacterium spp* was the least inhibited of all the organisms with 8.33±0.33mm zone of inhibition at 37°C and 45°C. *Proteus spp* was inhibited, but the effect was not temperature dependent. *B. subtilis* and *S. pyogenes* were resistant to the crude extract at all temperatures tested in neutral pH media. *B. subtilis, S. pyogenes,* and *E. coli* were totally inhibited at pH 10.0. The crude enzyme exhibited better activity against *Proteus spp.* at pH 10.0, but failed to inhibit the growth of *Corynebacterium spp*. Crude bromelain seems to be more effective in inhibiting gram positive bacteria than gram negative. Crude bromelain may be an effective antimicrobial agent against *E. coli* and *Proteus spp*.

Keywords: Crude Bromelain, Casein Digestion Unit, Bacteria

1. Introduction

Bromelain is a crude, aqueous extract from the stems and fruits of pineapples (*Ananas comosus*) derived from *Bromeliaceae* family [1]. In pineapple plant, bromelain is accumulated in the entire part with different extent and properties depending on its source. It is distinguished as either fruit bromelain or stem bromelain, with all commercially available being derived from the stem [2]. Bromelain is also present in pineapple wastes such as core, peel, crown, and leaves in relatively smaller quantities as compared to those in the stem [3,4]. Bromelain is a mixture of protein digesting enzymes called proteases and other several other substances in smaller quantities.

Bromelain is known for its clinical applications particularly modulation of tumour growth, blood coagulation, improvement of antibiotic action and anti inflammatory properties of therapeutic value [5]. Bromelain has been used for meat tenderization, solubilization of grain proteins, stabilization of beer, baking cookies, production of protein hydrolyzates, softening skins in leather and textiles [6]. It has been shown that bromelain is well absorbed after oral application and it has no negative impact on health after prolonged use [7]. Clinical studies have shown that bromelain may help in the treatment of several disorders. Bromelain exerts several inhibitory effects on platelette aggregation, bronchitis, angina pectoris, surgical traumas, sinusitis, thrombophlebitis and pyelonephritis. Moreover, it enhances absorption of drugs, especially antibiotics [8-10].

It is from the above background that this study was intended to investigate the antimicrobial effects of crude bromelain extract on some isolated microorganisms from fresh and overnight meat samples.

2. Materials and Methods

2.1. Crude Bromelain Extract Preparation

Fresh pineapple fruit was obtained from a local market

(Gamboru), Maiduguri, Borno State. The fruit was washed with distilled water, dried and peeled off. Seventy (70) grams of the fruit was homogenized using a blender by adding 40ml of cold phosphate buffer (pH 7.0, 0.1M). The mixture was filtered with cheese cloth and centrifuged at 4000rpm for 10minutes. The supernatant was collected and was referred to as 'crude bromelain extract' and was used for further experiments.

2.2. Protein Estimation

The concentration of protein in the crude extract was determined by previous method [11]. BSA was used as standard protein.

2.3. Enzyme Assay

The protease activity of bromelain was determined according to the casein digestion unit analytical method, and tyrosine was used as standard as described previously [6]. The reaction mixture contained 5ml of casein substrate prewarmed at 37ºC and 1ml of crude bromelain. After incubation at 37ºC for 10 mins, the reaction was stopped by adding 5ml of trichloroacetic acid. Precipitated protein was filtered and absorbance of the clear supernatant was read at 290nm. One unit of bromelain activity was defined as the amount of bromelain that will liberate 1µg of tyrosine after 1minute of digestion at 37ºC from a standard casein substrate solution at pH 7.0.

2.4. Source of Microorganisms

The microorganisms used for the study were isolated from fresh and overnight meat obtained from a local market, Kasuwan Shanu (a popular abattoir in Maiduguri.)

2.5. Isolation of Microorganisms

2.5.1. Culture Preparation

The streak plate method was used for plating. Briefly, a small piece of the meat was cut and smeared over one corner of the solid medium which was sufficiently dried. The wire loop was sterilized over a Bunsen flame, cooled and used to make parallel streaking from the main inoculated plate. The plates were then incubated at 37ºC for 24 hours and analyzed.

2.5.2. Preparation of Culture Media

The culture agar media used for the isolation were prepared according to the manufacturer's specification.

2.5.3. Identification of Microorganisms

The isolates were identified by standard methods [12]. Biochemical tests for sugar fermentation, catalase test, urease test, and Simon's citrate test were carried out for further identification.

2.6. Antimicrobial Activity of Crude Bromelain Extract

The antimicrobial activity of the crude extract was determined by the disc diffusion method. Nutrient agar plates were swabbed with the respective overnight cultures of the microbial isolates (diluted 1 in 10). Sterile 7mm diameter Whatman #1 filter paper discs impregnated with the crude extract (1.8mg/ml) were placed on the pre-seeded agar and incubated. Each media was incubated at three different temperatures, namely 25ºC, 37ºC, and 45ºC for 24hours. The zones of inhibition around the discs were measured.

To test for the antimicrobial activity of the crude extract at pH 4.0 and pH 10.0, sterilized acetate buffer (0.1 M, pH 4.0) and bicarbonate buffer (0.1 M, pH 10.0) were used respectively to dissolve the commercially prepared powdered nutrient agar. Sterile 7mm diameter Whatman #1 filter paper discs impregnated with 0.1M phosphate buffer (pH 7.0) was used as negative control.

3. Results and Discussion

Table 1 shows the isolated organisms and some biochemical characteristics of the isolates. Microorganisms isolated from the meat samples in this study have been earlier reported by many authors [13 -16]. From the results, the bacterial isolates were *Bacillus subtilis*, *Streptococcus pyogenes*, *Proteus species*, *Corynebacterium species* and two strains of *E. coli,* one of which fermented sucrose while the other did not. *B. subtilis*, *S. pyogenes*, and *Corynebacterium spp* were the gram positive bacterial isolates. *E. coli* and *Proteus spp* were gram negative. The genus, *Proteus* has been involved in the spoilage of meats, seafoods, and eggs. The presence of these bacteria in unrefrigirated foods in large numbers has made them suspects as a cause of food poisoning. *Corynebacterium spp* (particularly, *C. diptheriae*) may be transported by foods; it is a commensal in cow's udder and can be found in aseptically drawn milk and may be a cause of bovine mastitis. *E. coli* is generally regarded as part of normal flora of the human intestinal tract and that of many animals. Serotypes of *E. coli* have been implicated in human diarrhoeal diseases or food poisoning.

Table 2 shows the zones of inhibition (in mm) for the bacterial isolates incubated at various temperatures in neutral pH media. The result shows that *B. subtilis*, *S. pyogenes* and *E. coli* were resistant to the treatments. In general, temperature has minimal effect on the antimicrobial activity of the crude extract as well as the standard bromelain. This finding was in contrast to the work of Hanan [17], who reported the efficiency of bromelain to reduce *E. coli* and *Lysteria monocytogenes* increases with increase in temperature. However, at 25°C *E.coli* was found to be most susceptible to the crude enzyme just as with the standard bromelain. As the temperature was increased, the antimicrobial effect of crude bromelain on *E. coli* decreases slightly. Studies on the thermal stability of bromelain [18] revealed that bromelain from smooth cayenne pineapple showed higher retention of activity at low temperature and incubation at 40°C showed no loss of activity for up to 60minutes.

The effect of the crude extract on *Proteus spp.* was not temperature dependent. Inhibition of the growth of *Corynebacterium spp.* was the same at 37°C and 45°C, but

increased at 25°C (Table 2.). Bansode [19] reported that fresh pineapple fruit had antimicrobial effect against *E. coli* (6mm zone of inhibition by agar well diffusion method). Also, Hanan [17] reported the effectiveness of bromelain at concentrations of 1-4 mg/ml in reducing *E. coli* populations at 5°C, 25°C, and 35°C.

The gram positive bacteria, *B. subtilis* and *S. pyogenes* were resistant to both crude bromelain as well as the standard bromelain. This finding corroborates the works of Sparso [20] who concluded that bromelain is more efficient against gram negative than gram positive bacteria. More so, Khosropana [21] concluded that pineapple extract alone was not effective in growth inhibition of a species of *Streptococcus* (*S. sanguis*). *Bacillus subtilis* has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions.

From table 3, *B. subtilis, S. pyogenes,* and *E. coli* were totally inhibited at pH 10.0. The crude enzyme exhibited better activity against *Proteus spp.* at pH 10.0, but failed to inhibit the growth of *Corynebacterium spp*. Crude stem bromelain exhibited activity over a pH range of 4.5 to 9.8 [22]. Bhattacharya [7] reported that the primary component of bromelain is a sulfhydryl proteolytic fraction. The mechanism by which bromelain inhibits the growth of bacteria is not known. Bromelain probably may hydrolyze some peptide bonds present in the bacterial cell wall. Other components like phosphatases, glucosidases, peroxidases, cellulases, glycoproteins, carbohydrates and several other protease inhibitors are also present in crude bromelain [7].

Table 1. *Isolated microorganisms and some biochemical characteristics.*

ORGANISM	Hemolysis	Gram Reaction	Urease	Citrate	Xylose	Glucose	Lactose	Mannose	Sorbitol	Sucrose	Maltose
Bacillus subtilis	_	+ve	+	+	±	+	_	_	_	_	+
Streptococcus pyogenes	*β*	+ve	_	_	+	+	+	+	_	_	_
Escherichia coli	_	-ve	_	_	_	+	+	+	+	_	_
Escherichia coli	_	-ve	_	_	_	+	+	+	+	+	_
Proteus species	_	-ve	+	+	+	_	_	_	_	+	+
Corynebacterium species	_	+ve	_	+	_	+	+	_	_	+	+

Key: (β)= produces large zones of β hemolysis when cultured on blood agar plates, (-ve)= gram negative, (+ve)= gram positive, (-)= no reaction, (+)= positive reaction, (±)= 50% positive/ 50% negative.

Table 2. *Zones of inhibition (in mm) of the bacterial isolates at various temperatures in neutral pH media.*

Organism	Ampicloxacillin(2.5mg/ml)+crude extract(0.9mg/ml)			Standard bromelain (2.0mg/ml)			Crude bromelain extract (1.8mg/ml)		
	45ºC	37ºC	25ºC	45ºC	37ºC	25ºC	45ºC	37ºC	25ºC
BS	R	R	R	R	R	R	R	R	R
SP	R	R	R	R	R	R	R	R	R
EC	12.65±0.26^{c}	8.91±0.29	R	R	R	R	R	R	R
EC	29.33±0.66^{ac}	27.67±1.20^{b}	26.67±1.32^{c}	26.67±1.32	22.33±1.45	29.33±0.66^{ac}	19.00±0.85	22.23±1.53	24.00±1.53
PR	21.63±0.92^{abc}	24.65±0.33^{a}	22.00±0.58^{abc}	15.67±0.88	13.33±0.88	15.33±1.70	12.67±1.20	14.67±1.53	13.67±0.88
CR	14.23±0.53^{a}	13.33±0.35^{c}	13.67±0.81	11.67±0.83^{c}	9.33±0.33	11.67±0.83^{c}	8.33±0.33	8.33±0.33	9.33±0.33

Values are mean ± SEM of triplicates, R= Resistant, BS=Bacillus subtilis, SP=Streptococcus pyogenes, EC=Escherichia coli, PR=Proteus spp, CR=Corynebacterium spp, Superscripts a, b, and c indicate significant differences for $p<0.001$, $p<0.01$, and $p<0.05$ respectively.

Table 3. *Zones of inhibition (in mm) of the bacterial isolates at 37ºC in alkaline and acidic medium.*

Organism	Ampicloxacillin (2.5mg/ml)+ crude extract(0.9mg/ml)		Standard bromelain (2.0mg/ml)		Crude bromelain extract(1.8mg/ml)	
	pH 10.0	pH 7.0 pH 4.0*	pH 10.0	pH 7.0 pH4.0*	pH 10.0	pH 7.0 pH 4.0*
B. subtilis	TI	R	TI	R	TI	R
S. pyogenes	TI	R	TI	R	TI	R
E. coli	TI	8.91±0.29	TI	R	TI	R
E. coli	32.67±1.78^{ab}	27.67±1.20^{a}	9.67±0.88	22.33±1.45^{a}	8.33±0.33	22.23±1.53^{a}
Proteus spp.	40.33±1.45^{a}	24.65±0.33^{a}	29.67±1.45^{a}	13.33±0.88	18.67±1.20	14.67±1.53
Corynebacterium spp.	29.67±1.45^{a}	13.33±0.35^{c}	14.67±1.02^{bc}	9.33±0.33	R	8.33±0.33

Values are mean ± SEM of triplicates, Key: R= Resistant, TI= total inhibition of the growth of the organism, Superscripts a, b, and c indicate significant differences at $p<0.001$, $p<0.01$ and $p<0.05$ respectively. * Not tested. The nutrient agar was not stable in the buffer, it does not solidify readily.

4. Conclusion

The study suggested that crude bromelain may be effective as antimicrobial agent against *E. coli*, and *Proteus spp*. A specific strain of *E. coli*, *Streptococcus pyogenes*, and *Bacillus subtilis* were resistant to crude bromelain. The crude extract was most potent at 25°C and 37°C in neutral pH medium against *E. coli* and *Proteus spp*. respectively. The crude enzyme showed better activity against *Proteus spp.* at pH 10.0. Combination of the crude bromelain and an antibiotic had most effect than either standard or crude bromelain.

References

[1] Muntari, B., Maizirwan, M., Mohammed, S. J., Azura, A., Hamza ,M. S. (2013). Kinetic studies on recombinant stem bromelain. *Advances in Enzyme Research*, 1: 52-60

[2] Gautam, S. S., Mishra, S. K., Dash, V., Amit, K. G. and Rath, G. (2010). Comparative study of extraction, purification and estimation of bromelain from stem and fruit of pineapple plant. *Thai Journal of Pharmaceautical Sciences,* 34: 67-76

[3] Hebbar, H. U., Sumana, B. and Raghavarao, K. S. M. S. (2008). Use of reverse micellar systems for the extraction and purification of bromelain from pineapple waste. *Bioresource Technology*, 99: 4896-4902

[4] Noryawati, M., Elizabeth, R., Jessie, G. P. M., Barbara, O. V. and Thenawidjaja, S. (2013). Quality and quantity of bromelain in some Indonesian pineapple fruits. *International Journal of Applied Biology and Pharmaceutical Technology*, 4: 235-240

[5] Nazirah, K. Z., Zainal, S., Noriham, A. and Normah, I. (2013). Efficacy of selected purification techniques for bromelain. *International Food Reseach Journal,* 20(1): 43-46.

[6] Ketnawa, S., Sai-ut, S., Theppakorn, T., Chaiwut, P. and Saroat, R. (2009). Partitioning bromelain from pineapple peel (Nang Lae cultiv.) by aqueous two phase system. *Asian Journal of Food and Agro-industry,* 2(04): 457-468

[7] Bhattacharyya, B. K. (2008). Bromelain: an overview. *Natural Product Radiance,* 7(4): 359-363.

[8] Bintang, N. T., Zhang, W., Shi-ying, X. and Wenbin, Z. (2008). Therapeutic applications of pineapple protease (bromelain): a review. *Pakistan Journal of Nutrition* , 7(4); 513-520

[9] Bromelain monograph. (2010). *Alternative Medicine Review.* 15: 361-368.

[10] Maurer, H. R. (2001). Bromelain: biochemistry, pharmacology and medicinal use. *CMLS Cellular and Molecular Life Sciences*, 58(9): 1234-1245

[11] Bradford, M.M.A. (1976), Rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein- dye binding. *Analytical Biochemistry*, 72: 248-254

[12] Buchanan, R. F. and Gibbon, N. E. (1974). Bergey's manual of determinative bacteriology. 8TH edition. *The Williams and Wilkins Co. Baltimore*

[13] Ashok, K., Varun, B., Shikha, V., Gaurav, S., and Sushil, k. (2011). Isolation and characterization of microorganisms responsible for different types of food spoilages. *International Journal of Research in Pure and Applied Microbiology*. 1(2): 22-23.

[14] Egbebi, A. O., and Seidu, K. T. (2011). Microbiological evaluation of suya (dried smoked meat) sold in Ado and Akure, South-Western Nigeria. *European Journal of Experimental Biology.* 1(4): 1-5

[15] Moshood, A. Y., Tengku, H., Abdul T. A., and Ibrahim, H. (2012). Isolation and identification of bacteria associated with balangu (roasted meat product) sold in Bauchi, Nigeria. *IOSR Journal of Pharmacy and Biosciences*, 2(6): 38-48.

[16] Uzeh, R. E., Ohenhen, R. E. and Adeniyi, O. O. (2006). Bacterial contamination of tsire-suya, a nigerian meat product. *Pakistan Journal of Nutrition*, 5(5): 458-460.

[17] Hanan, E., Inyee, H., Hesham, N., James, R. and Paul, D. (2013). Bactericidal effects of meat tenderizing enzymes on *E. coli* and *Lysteria monocytogenes*. *Journal of Food Research,* 2(1): 8-18

[18] Rungtip, J. and Sanguansri, C. (2010). Effect of temperature on the stability of fruit bromelain from smooth cayenne pineapple. *Kasetsar Journal (Natural Science)*, 44; 943-948

[19] Bansode and Chevan (2013). Evaluation of the antimicrobial activity and phytochemical analysis of papaya and pineapple fruit juices against selected enteric pathogens. *International Journal of Pharmaceutical and Biosciences,* 4(2): B-1176

[20] Sparso, H. M. and Moller, S. M. (2002). Proteolytic enzyme as antimicrobial agents and incorporation of hydrophobic additives into thermally compacted soy protein-based films. Research thesis at Clemson University exchange with Technical University of Denmark.

[21] Khosropanah, H., Bazargani, A., Ebrahim, H., Eftekhar, K., Emani, Z., Esmailzadeh, S. (2012). Assessing the effect of pineapple extract alone and in combination with vancomycin on *Streptococcus sanguis*. *Judispur Journal of Natural Pharmaceutical Products,* 7(4): 140-143.

[22] Jeung A. (1980). Encyclopaedia of common natural ingredients used in foods, drugs, and cosmetics. John Wiley and Sons, New York. Pp 74-76.