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Evaluation of the Antibacterial activity of Ananas comosus (Pineapple) extract against selected gram-negative bacteria

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ABSTRACT

Pineapples are a tropical fruit that present with a wide array of health and beauty benefits. They are not only valued for their sweet taste, but have been used for centuries to treat digestion problems and inflammation. Studies have shown that bromelain, the enzyme found in pineapples, can reduce swellings, bruising, healing time, and pain associated with injury and surgical intervention. Bromelain is currently being used to treat and reduce inflammation from tendinitis, sprains, strains, and other minor muscle injuries as well as swelling related to ear, nose and throat surgeries or trauma. The present study was aimed at determining the antimicrobial activity of pineapple extract against selected gram-negative bacteria. The aim of the study was to evaluate the antibacterial activity of both stem and fruit pineapple crude extract against selected gram-negative bacterial pathogens notably *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi.* The antibacterial activity was carried out by agar well diffusion technique against the bacterial pathogens and the zone of inhibition were measured in mm diameter and the minimum inhibition concentration was calculated on every organism. In the study, pine apple extract was found to be effective against the all organisms tested when compared to control (ciprofloxacin), the stem extract showed highest zone of inhibition in *P. aeruginosa* 22mm, *S. typhi* 17mm and *E. coli* 20mm and highest minimum inhibition concentration was 0.125g/ml, 0.25g/ml and 0.125g/ml for *P. aeruginosa, S. typhi* and *E. coli* respectively. For fruit crude extract, highest zone of inhibition in *P. aeruginosa* was 13mm, *S. typhi* 7mm and *E. coli* 12mm and highest minimum inhibition concentration was 0.25g/ml, 0.25g/ml and 0.125g/ml for *P. aeruginosa, S. typhi* and *E. coli* respectively. Generally, the stem crude extract has a high antimicrobial effect than the fruit crude extract on the organisms tested and it was found that *E. coli* and *P. aeruginosa* trended to be more susceptible than *S. typhi*. The finding that pineapple juice has antibacterial effect serves to suggest that there is a potential in discovering novel antimicrobial agents from the pineapple plant. This however needs further investigation.

Keywords: Antibacterial activity, antimicrobial activity, Pineapples

INTRODUCTION

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric time (Ahn, 2017).

This is because they produce a large number of diverse bioactive compounds for therapeutic value and high concentrations of phytochemicals, which may protect against free radical damage (Suffredini *et al.* 2004). According to the World Health Organisation (WHO), between 65% and 80% of the populations of developing countries currently use medicinal plants as remedies. Pineapple has been used as part of traditional folk medicine since ancient times and it continues to be present in various herbal preparations (Rathnavelu *et al.* 2016). The development of new products from natural sources is also encouraged because it is estimated that of the 300,000 plant species that exist in the world, only 15% have been evaluated to determine their pharmacological potential (Palhares *et al.* 2015). There has been an increased interest in antimicrobial agents of plants origin due to the resistance that microorganisms have developed against traditional antibiotics.

Antibiotic resistance has been referred to as "the silent tsunami facing modern medicine". It has been the topic of numerous international health summits and political summits and the rise of multidrug resistance in Gram negative bacteria (MDR-GNB) has become a particularly serious challenge for health care professionals (Shaikh *et al.* 2015). Existence of antibiotic resistance due to the extended spectrum of beta lactamase producing gram negative is a major global public health problem and has been reported in all regions of the world (Shaikh *et al.* 2015) and increase in antibiotic resistant bacteria is larger due to the generalized use of antibiotics in medicine, in animal care and in agriculture. The treatment of serious bacterial infections in clinical practice is often complicated by antibiotic resistance (Slama, 2008). Based on clinical experience, most of the pathogens have been resistant to antibiotics due to their virulence factors. Resistance rates are increasing among several problematic Gram-negative pathogens that are often responsible for serious nosocomial infections, including *Acinetobacter spp*., *Pseudomonas aeruginosa*, and (because of their production of extended spectrum β-lactamase (Slama, 2008). A hospital in Bangladesh reportedly found 75% of *Salmonella typhi* to be resistant to Ciprofloxacin. In the face of such adversity, it is important that a search for other alternatives is established. This search is now focused to plants and their antimicrobial properties (Hossain *et al.* 2015).

The researchers have been initiated in the search of new antimicrobial agents in order to encounter the resistant bacteria. One such enzyme is bromelain which is a component of pineapple juice. Bromelain is a mixture of proteolytic enzymes and is under preliminary research for a variety of clinical disorders, but to date has not been adequately defined for its effects in the human body. Bromelain is unsafe in some circumstances like pregnancy, allergy and anticoagulation therapy (Hossain, *et al.* 2015). Recently, researchers had a suggestion that bromelain may have antibacterial and antiviral activity and several studies have shown that bromelain inhibited certain bacteria and viruses in-vitro (Loon, 2018). Bromelain is a mixture of Thiopepdidases, such asananain and Comosain, Phosphatases, Glucosidases, Peroxidases, Cellulases, Glycoproteins, Proteinase inhibitors, such as cystatin (Ahamed *et al.*2016).

Some of the medicinal uses of bromelain include antithrombotic and anti-inflammatory activity, treatment of cardiovascular diseases, removal of dead tissue (debridement) of severe burn and the proteolytic enzymes contained in bromelain have been investigated in Europe to evaluate the efficacy in breast and plasmacytoma cancer patients (Beuth, 2018). It has been shown that bromelain is well absorbed after oral application and it has no negative impact on health after prolonged use. Bromelain enhances absorption of drugs, especially antibiotics and clinical studies have shown that bromelain may help in the treatment of several disorders. Bromelain exerts several inhibitory effects on platelet aggregation, bronchitis, angina pectoris, surgical traumas, sinusitis, thrombophlebitis and pyelonephritis (Ali *et al.*2015). Bromelain can modify the permeability of organs and tissues to different drugs. In humans, bromelain has increased the action of antibiotics and results by increasing the blood and tissue levels of tetracycline and amoxicillin when they are administered concurrently with Bromelain (Nadu, 2016). Bromelain supplementation has proved some antibacterial activity by protecting animals against diarrhea caused by bacterial enterotoxins (Mynott, *et al.* 1997), anti-fungal activity by stimulating phagocytosis and respiratory burst killing of *Candida albicans* when incubated with trypsin in vitro. In its addition to its ability to counter effects of particular intestinal pathogens and its synergism with antibiotics, these two mechanisms are indicative of bromelain benefits on specific bacterial

infections (Rathnavelu *et al.* 2016). The aim is to evaluate the antibacterial activity of pineapple extract on selected gram-negative bacteria.

MATERIAL AND METHODS

Research design

This was an experimental study that involved random purchasing of 2 raw and 2 ripe pineapples (*Ananas comosus)* from kalerwe market, Kampala district during the month of April. The fruits were brought to college of veterinary medicine, Animal resources and biosecurity Makerere University where they were finally homogenized. The data obtained was entered in Microsoft excel and analyzed using Special Statistical Package for Social Scientists (SPSS) 2018 version.

Sample collection

Ananas comosus used in this study was purchased from Kalerwe market in Kampala district, Uganda which is one of the biggest local markets, packaged in a sterile container and transported to the microbiology laboratory.

Sample processing

The fruits were selected and rinsed thoroughly with distilled water, peeled with sterile stainless knife and cut into small pieces of about 3-4 mm thick. The diced pineapple stem and fruit was aseptically loaded into a blender and quashed.

Laboratory methods

Preparation of the stem and fruit crude extracts

The pineapples were selected and rinsed thoroughly with 70% ethanol solution, peeled off, cut into small pieces of 4 mm thick with sterile stainless knife both the stem and fruit and then weighed. The weighed mass was found to be 300 gm. Juice was collected from the fresh pineapple fruit part by quashing using a sterile blender. Fresh pineapple stem parts were collected, washed with 70% ethanol solution and made into small cubes. The weighed mass was 80 gm and juice were extracted by quashing using a sterile blender. The filtrate obtained was called "crude extract" of pineapple.

Preparation of bacterial cultures

Standard strains of E. coli, *S. typhi* and *P. aeruginosa* were obtained from Mulago Hospital Microbiology laboratory, Kampala, Uganda and sub cultured on XLD agar and King's agar. Inocula of each bacterium were suspended in 5ml of Bain Heart infusion broth and incubated at 37^{o}C for 24hrs. The turbidity of the sub cultured microorganisms were adjusted with sterile distilled water using 0.5 Mc Farland as standard representing approximately 1.5 × 10^{8} per ml 0f the test organism.

Determination of antibacterial activity

Agar well diffusion Assay

Antibacterial efficacy was tested using the agar well diffusion Assay. Plates were prepared by pouring 20 ml Mueller-Hinton (MH) agar into sterile petri dishes and allowed to cool in a sterile environment. Inocula of each bacteria strain was suspended in 5ml of Bain Heart infusion broth and incubated at 37^{o}C for 24hrs. Each plate was inoculated with 0.1 ml of bacteria culture directly from the 24-hour Brian Heart infusion broth culture and diluted to match 0.5 McFarland standard representing approximately 1.5×10^{8} per ml of test organism. Wells of 6mm diameter and 5mm depth were made on the solid agar using a sterile glass borer. 50µl of each extract for stem part and fruit part were dispensed into respective wells using a micropipette and 10µl water was used as a negative control. Ciprofloxacin, an antibiotic disc was used as a positive control. All the tests were run in triplicate to ensure quality results. The set was incubated for 24hr at 37°C. 24hr later, the zones of inhibition were measured using a divider and results will be recorded in millimeters.

Determination of the Minimum Inhibitory Concentration of *Ananas comosus* extract

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that inhibits any visible bacterial growth on culture plates. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against microorganisms and was determined by broth dilution method. Three sets of six test tube containing 900µl nutrient broth were prepared for three test microorganisms. Then 100µl of each concentration of fruit juices were added into the respective test tubes and serial diluted to fifth dilution by tenfold dilution factor. After this step 0.1ml test pathogen suspension were inoculated into respective labeled test tube. After inoculation, the test tubes were kept in a shaker incubator for overnight at 37^{o}C and results were

observed in the form of turbidity and O.D. observed at 600nm on U.V. Spectrophotometer.

Determination of Minimum bactericidal concentration of *Ananas comosus* extract

The minimum bactericidal concentration is the lowest concentration of an antibacterial agent required to kill a particular bacterium (*S. typhi, E. coli and P. aeruginosa)*. MBC was identified by determining the lowest concentration of antibacterial agent (pineapple extract) that reduces the viability of the initial bacterial inoculum by greater or equal to 99.9%. The minimum bactericidal concentration was determined sub culturing the tubes representing MIC and they were incubated at 37^{o}C for 24 hrs. The following day the plates were examined to determine the concentration that completely eliminated the growth of bacteria.

Data analysis

Microsoft excel was used to enter and capture data and thereafter data was exported to SPSS for further analysis.

Ethical considerations

Permission was sought from the College of Veterinary Medicine Animal Resources and Biosecurity and Bioethics and Research Committee Makerere University.

Limitations and mitigations of the study

The contamination of the research personnel and samples was a limitation and was mitigated by thorough disinfection of the hands and work benches using a disinfectant before collecting and analyzing samples. Interference limitation with samples in the laboratory because of many students working from the laboratory and this was mitigated by putting a caution and proper labelling.

Table 1: Antimicrobial activity of Pineapple crude extract (Zone diameter in mm)

	Zone diameter(mm)		
Test organism	**Crude stem extract**	**Crude fruit extract**	**positive control**
Pseudomonas aeruginosa	22.25±1.75	13.25±2.09	33.00±0.00
Salmonella typhi	17.58±2.29	7.92±2.60	30.00±0.00
Escherichia coli	20.25±1.89	12.25±1.84	14.00±0.00

Table 2: Minimum inhibitory concentration (MIC) of Pineapple Crude Extract

	Minimum Inhibition concentration (g/ml)	
Test organism	**Crude stem extract**	**Crude fruit extract**
Pseudomonas aeruginosa	0.125	0.25
Salmonella typhi	0.25	0.25
Escherichia coli	0.125	0.125

Table 3: Minimum Bactericidal concentration (MBC) of Pineapple Crude Extract

	Minimum bactericidal concentration (g/ml)	
Test organism	Crude stem extract	Crude fruit extract
Pseudomonas aeruginosa	0.25	0.25
Salmonella typhi	0.25	0.25
Escherichia coli	0.125	0.25

Table 4: Statistical analysis of ZOI results

Variable	**t**	**df**	**p value**
Zone of inhibition for *E. coli* (mm)	7.433	10	0.000
Zone of inhibition for *S. typhi* (mm)	6.84	10	0.000
Zone of inhibition for *P. aeruginosa* (mm)	8.077	10	0.000

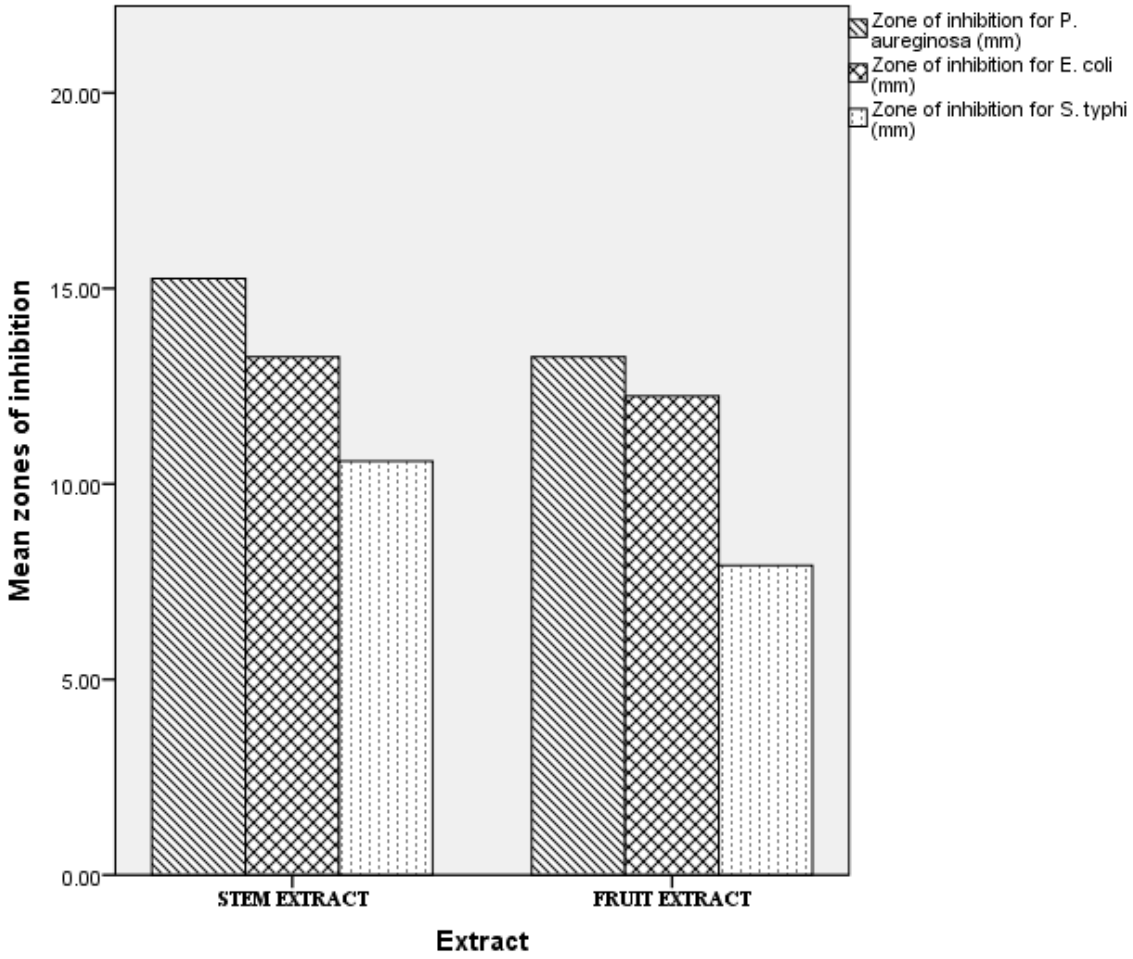

Figure 1: A graph showing mean zones of inhibition of crude extracts

DISCUSSION

The results of the present study have shown that the pineapple extract has antimicrobial effect towards all the organisms tested. From the agar well diffusion assay, the results showed that *P. aureginosa* was the most sensitive bacteria compared to others as indicated by the lowest inhibitory concentration (MIC) of the extract (0.125g/ml) and the largest inhibition zone 22.25±1.75 mm when the stem extract was applied as shown in Table 1 and 2. Whereas the lowest concentration that could inhibit *E. coli* and *S. typhi* was (0.125 g/ml) and (0.25g/ml) with zones of inhibition 20.25±1.89 and 17.58±2.29 respectively. From the results of one-way ANOVA, the *P-values* obtained were all less than 0.05 at 95% confidence interval for each of the plant extracts on the test organisms as shown in table 4. This indicated a statistically significant difference in the ZOI of the different extracts (stem and fruit) on the test organisms. The variation in the ZOI could be due to the varying concentration of active components in different parts of the plant. There is therefore an indication that the extract possesses natural substances that can inhibit the growth of some microorganisms. Natural substances have demonstrated antibacterial action mainly because most plants used in alternative medicine are composed of flavonoids, which act on bacterial cells disrupting the cytoplasmic membrane and inhibiting the enzymatic activity (Nadu, 2016).

The exact antimicrobial mechanism of pineapple extract against gram negative bacteria is still not clearly known. Previous studies suggested that the active substance working against gram negative bacteria are majorly bromelain and saponins, while flavonoids and polyphenols are more potent in inhibiting gram-positive bacteria. Bromelain and saponins act on bacteria cell walls and membranes in a mechanism that might have not been recognized by the various bacteria that were tested in the study. Bromelain is a proteolytic enzyme and plays a role in the breakdown of proteins, as we know protein is one of the essential components in bacterial membrane. Bromelain is hypothesized to induce protein breakdown in bacteria membrane, causing injury and cell death. The exact mechanism of how bromelain inhibits the growth of gram-negative bacteria is still not full identified. Studies suggest that bromelain operates through associated mechanism in weakening of outer membrane in gram negative bacterial which contains proteins. Bromelain are thought to disintegrate protein in surface membrane which eventually weakens the cell wall, leads to cell leakage, swells the cell, and damages the cell (Eshamah *et al.* 2013)

Saponins increase the permeability of the bacteria cell membrane, causing alteration of structure and function of the membrane, disrupting the surface tension of the cell wall, then allowing antibacterial substances to easily enter the cells and interfere the cell metabolism while denatures proteins on membrane so the cell membrane will be lysis. Saponins selectively interacts with cholesterols on cell membrane, leaving a hole in the membrane (Seeman *et al.* 1973).

In the broth dilution assay as shown in table 2, different concentration of the pineapple extract (0.1 g/ml – 0.03125 g/ml) were applied in order to observe the minimum inhibition (MIC) and minimum bactericidal concentration (MBC) of extract. It seems that *E. coli* and *P. aureginosa* were more susceptible to the pineapple extract, than *S. typhi* indicating by the lowest MIC of 0.125 g/ml which is similar to those results obtained from the agar well diffusion assay. A study conducted by (Praveen *et al*. 2014) showed minimum concentration of 0.3125g/ml for *E. coli* which showed effectivity of the extract at the lowest concentration.

In addition, lethally effect was observed in all the tested bacteria with 0.125 g/ml for *E. coli* and 0.25 for both *P. aureginosa* and *S. typhi* which is contradictory to the study conducted by (Ajibade *et al.* 2015), pineapple extract eliminated Pseudomonas aeruginosa at concentration of 0.2g/ml. The lethally effect might be due to the effect from some bioactive compounds in the pineapple extract, particularly phenolic compounds that causes changes in permeability of the cell structure. Those changes could lead to leakage of some intracellular cell contents or permission of those bioactive compounds to the cell causing to the cell death.

CONCLUSION

Pineapple crude extract showed antibacterial activity against all organisms tested. In stem crude extract, highest zone of inhibition in *P. aureginosa* was 22mm, *S. typhi* 17mm and *E. coli* 20mm and highest minimum inhibition concentration was 0.125g/ml, 0.25g/ml and 0.125g/ml for *P. aureginosa, S. typhi* and *E. coli* respectively. For fruit crude extract, highest zone of inhibition in *P. aureginosa* was 13mm, *S. typhi* 7mm and *E. coli* 12mm and highest minimum inhibition concentration was 0.25g/ml, 0.25g/ml and 0.125g/ml for *P. aureginosa, S. typhi* and *E. coli* respectively. Generally, the stem crude extract has a high antimicrobial effect than the fruit crude extract on the organisms tested and it was found that *E. coli* and *P. aureginosa* trended to be more susceptible than *S. typhi*. The finding that pineapple juice has antibacterial effect serves to suggest that there is a potential in discovering novel antimicrobial agents from the pineapple plant. This however needs further investigation.

RECOMMENDATION

- Toxicity studies should be done to establish safety levels of pineapple for use as an antibiotic.
- Qualitative and quantitative phytochemical analysis of pineapple extract be be done since the study involved screening.
- Different parts of the plant should be screened for antibacterial activity since this study only concentrated on fruit and stem.
- Similar tests should be done using the extracts like alcohol extract, methanoic extract and other

screening tests be done on the plant for maximum recovery of active compounds.

Conflicts of interest: The authors stated that no conflicts of interest.

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