



Antimicrobial activity of Crude extraction of Bromelain enzyme from waste peel of pineapple against pathogenic bacteria

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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. This study is aimed to collect different morphotypes of *A. Comosus* from public market to assess the bacterial composition and antibacterial properties using standard procedures. Bromelain is a protease, commercially derived from pineapple fruit or stem. Bromelain also showed significant anticancer activity against breast cancer cells, melanoma cells, human epidermoid carcinoma cells, malignant peritoneal mesothelioma cells and mammary carcinoma cells. The major anticancer mechanism of bromelain is by inhibiting cancer cell proliferation and inducing differentiation of leukemic cells. Pineapples (*Ananas comosus*) have exceptional juiciness and a vibrant tropical flavor that balances the tastes of sweet and tart. It has a wide variety of health benefits. The aim of the study is to evaluate the antimicrobial activity of pineapple peel extract on against selected bacterial pathogens.

Keywords: Phosphate buffer, pineapple peel, ethanol etc.

INTRODUCTION

Pineapple is a favourite of the lovers of fruits in its fresh form, sweetness as well as its preserve like jams and jellies. It is the 3rd most important fruit crop in the tropical region of the world after banana and citrus fruit in terms of production (Pooja et al., 2007). The pineapple is a rich source of manganese, which helps build and maintain bone strength and it also has plenty of vitamin C. It also contains bromelain, an enzyme extract, studies has shown that the enzyme found in pineapples can reduce swelling, bruising, healing time and pain associated with injury and surgical intervention (Thanish et al., 2016). In the scientific binomial *Ananas Comosus*, ananas, the original name of the fruit, comes from the Tupi word nanas, meaning "excellent fruit" (Davidson et al., 2008).

Bromelain has been used widely in food, medical, pharmaceutical and cosmetic industries and other industries as well. In the food industry, it is used for meat tenderization, grain protein solubilization, beer clarification, baking cookies and protein hydrolysate production (Walsh, 2002). For medical and pharmaceutical industries, bromelain is well known for clinical and therapeutic applications, particularly for modulation of tumor growth, third-degree burns, improvement of antibiotic action, and as a drug for the oral systemic treatment of inflammatory, blood coagulation related and malignant diseases and digestion (Maurer, 2001). Bromelain, the proteolytic enzyme, is found in the tissues of the plant family Bromeliaceae of which pineapple (*Ananas comosus* L. Merrill) is a well-known source. It is also found in pineapple wastes such as cores, peels and leaves in relatively smaller quantities as compared to stems and fruits (Maurer, 2001; Sriwatanapongse et al., 2000; Umesh et al., 2008).

MATERIAL AND METHODS

Collection of fruit :-

Fresh pineapple fruit was collected from a local market in akola city.

Preparation of fruit extraction:-

The fruit was washed with distilled water, dried and peel off. Then Seventy (70) Grams of the fruit was homogenized using our blender by adding 40 ml of cold phosphate Buffer. The mixture was filtered with muslin cloth and centrifuged at 4000 rpm for 10 minutes. The supernatant was collected and was referred to as 'crude bromelain extract' and was used for further experiments.

Collection of clinical sample:-

Multiple urine, blood and pus samples were collected from GMC Akola. The urine, blood and Pus samples collected are specifically from the patients suffering from various types of infection.

Isolation of clinical samples:-

The collected urine, Blood and pus samples were inoculated on nutrient agar plates. The nutrient agar was prepared by adding 28 gm of nutrient agar in 1000 ml of distilled water. It was prepared in conical flask, and then the media was heated to dissolve the powdered media completely. It was then sterilized in autoclave at 121°C for 15 min and then allowed to cool. After that the media was poured in sterile Petri plates and then allowed to solidify.

Inoculation of samples:-

A loopfull of urine, blood and pus samples were inoculated on the separate nutrient agar plates. Inoculated plates were kept for incubation in incubator for 37°C for 24 hrs.

Antimicrobial Activity :-

The bacterial strains were used for this activity are *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhi* and *proteus vulgaris* were taken. To test the antimicrobial activity test, Nutrient agar is prepared by adding 28 gms of nutrient agar in 1000 ml of distilled water or by Muller Hinton agar by adding 38 gms in 1000 ml of distilled water. It is then sterilized in autoclave at 121°C for 15 min and then allowed to cool. Cooled media is then poured in Petri plates.

Nutrient broth is prepared by adding 26 gm Nutrient broth in 1000 ml distilled water. The prepared Nutrient broth is then sterilized in autoclave at 121°C for 15 min and then allowed to cool. After the broth gets cooled, loop full culture of desired organism is added in the broth And kept for incubation overnight.

Then make lawn of the different bacterial broth on the agar plate with sterile cotton swab. After making a lawn of culture, Each plate is made with wells that are filled with Extraction. Each plate was incubated at three different temperatures, namely 25°C, 37°C, and 45°C for 24 hours. The zones of inhibition around the wells were measured.

RESULTS AND DISCUSSION

Result :-

Conformation of the pathogenic micro-organisms. The pathogenic micro-organisms were confirmed as *E.coli*, *Pseudomonas*, *Klebsiella*, *Proteus* and *Salmonella* on the basis of gram staining, microscopy and sugar fermentation, biochemical tests and their results are given in table 5.1 and 5.2

Antimicrobial activity of well diffusion method

The antimicrobial activity of phosphate buffer and ethanol extract pineapple peel against five gram-ve pathogenic microorganisms was carried out by well diffusion method.

It was found that the peel extract showed good inhibitory activity on some pathogenic microbes tested.

The results are described as follows

Antimicrobial activity of peel extract against pathogenic microorganisms result shows that *Escherichia coli*, *Klebsiella pneumonia* was studied under invitro condition with different solvents extracts.

Among the extracts tested, ethanol extract recorded highest antimicrobial activity against the pathogenic microorganisms.

Phosphate buffer extract where intermediary in their ability.

Interpretation of result of minimal inhibitory concentration (MIC)

Minimal inhibitory concentration of pineapple peel extracts were tested against two pathogenic microorganisms viz., *Escherichia coli* and *Klebsiella pneumonia* on the basis of results obtained by well diffusion method.

The two isolates were susceptible to the solvent extracts when compared to other pathogenic microorganisms.

Escherichia coli were found to be more susceptible to the plant extracts viz., pineapple peel extracts, since their growth was inhibited at relatively lower concentration *Klebsiella*.

The Pineapple peel extracts inhibited the tested pathogenic microorganisms viz., *E.coli* And *Klebsiella pneumonia* with highest concentration to a maximum level than other extracts.

Antimicrobial activity of pineapple peel extracts against *Escherichia coli* and *Klebsiella pneumonia*.

Micro-organism used	Concentration of Extract mg/ml	Average of Inhibition Zone (mm)
<i>Escherichia coli</i>	100	18
<i>Klebsiella pneumonia</i>	100	12

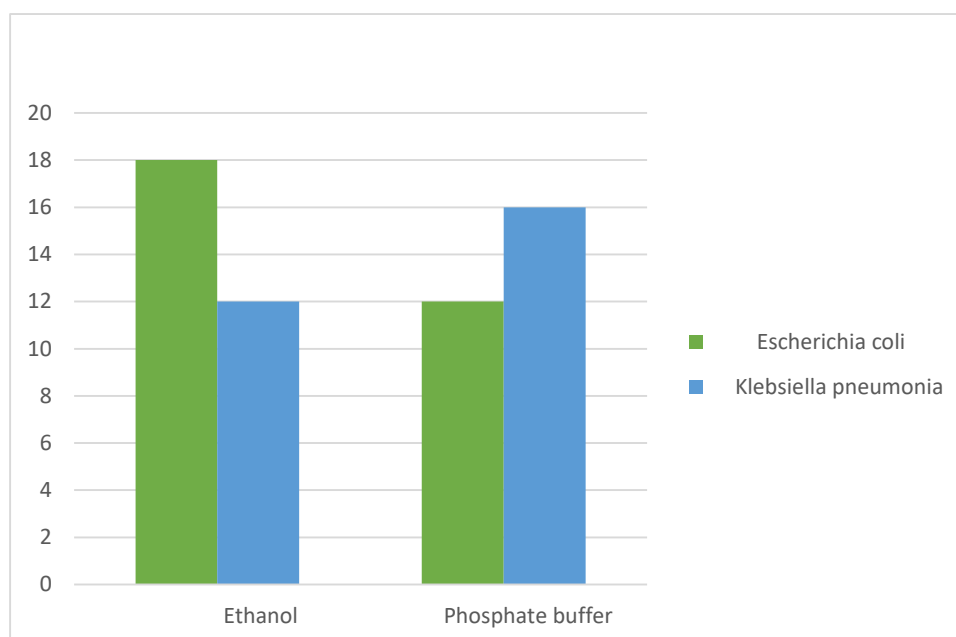
Antimicrobial activity of pineapple peel extracts (mm)

Name of organism	Ethanol	Phosphate buffer
<i>Escherichia coli</i>	18	12
<i>Klebsiella pneumonia</i>	12	16

Discussion:-

Table no. 5.3 shows that the zone of inhibition (in mm) for the bacterial isolates incubated at various temperatures in neutral pH media. The result shows that *Escherichia coli*, *Klebsiella pneumonia* were resistant to the treatments. In general temperatures has minimal effect on the antimicrobial activity of the crude extract as well as the standard bromelain. 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.

Graph 5.1: Graphical representation of Antimicrobial activity of pineapple peel extracts



Antimicrobial Activity of pineapple peel extract against two pathogenic microorganisms viz., E coli and Klebsiella pneumonia :-



CONCLUSION

The Study suggested that crude extraction of pineapple peel extracts may be effective as antimicrobial agent against E. Coli, and Klebsiella pneumonia. The crude extraction showed better activity against E.coli.

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