

## ORIGINAL ARTICLE

**Investigation of The Antibacterial Effects of Papaya (*Carica papaya*) Seeds on Three Pneumonia Causing Bacteria**

Dawit Dikasso<sup>1\*</sup>, B. Pharm, Hirut Lemma<sup>1</sup>, MSc, Kelbessa Urga<sup>1</sup>, MSc, Chernet Ambaye<sup>1</sup>, B. Pharm, Ashenafi Ayele<sup>1</sup>, Pharm Tech, Kidist Yersaw<sup>1</sup>, Lab Tech.

## ABSTRACT

**BACKGROUND:** *Papaya (Carica papaya)* is one of the herbal remedies, which has recently become a subject of research focus. It is used in traditional medicine for a variety of purposes besides its common anthelmintic, carminative, diuretic, oxytotoxic and ant infective effects. In this paper we report the antibacterial effects of aqueous papaya seed extracts against three pneumonia causing bacteria.

**METHODS:** The study was conducted between November 2000 and June 2001. The antibacterial activity of the crude aqueous extract of papaya (*C. papaya*) seeds was investigated against specific pneumonia causing bacterial by an agar dilution technique. The growth or inhibition of the standard test microorganisms as well as clinical isolates of *Streptococcus pneumoniae*, *Staphylococcus aureus* & *Klebsiella pneumoniae* were determined in growth media. Fresh ripe papaya fruits were purchased from a local market and the seeds were collected, thoroughly cleaned with distilled water, sun dried, powdered, sieved with a mesh and macerated. The macerate was filtered through No.1, 18.5 cm Whatman filter, cooled immediately to -20 °C and lyophilized. The powder obtained was weighed, diluted with distilled water and the concentration determined.

**RESULTS:** All the test organisms were successfully inhibited by 11.8 mg/ml of the extract except *Streptococcus pneumoniae* standard test organism which was inhibited by 18.38 mg/ml of media, indicating that *Streptococcus pneumoniae*, clinical isolate, is the least sensitive.

**CONCLUSION:** *Papaya (C. papaya)* seed could be used as an effective antibacterial agent. Further purification and extraction of the active principle would give a true antibacterial activity comparable to standard antibiotics. Nevertheless, clinical trial on the effects of the seed is essential before advocating large-scale therapy.

**KEY WORDS:** *Carica papaya*, antibacterial, papaya, pneumonia

<sup>1</sup>Ethiopian Health and Nutrition Research Institute, P. O. Box 1242. Addis Ababa, Ethiopia

\* Corresponding author

## INTRODUCTION

Pneumonia may be caused by different class of microorganisms of which bacterial agents account for more than 50% of acute respiratory infection cases. The most common bacteria are *Streptococcus pneumoniae* (1). Like in other developing countries, it has been shown that children in Ethiopia are seriously affected by communicable diseases, among which acute respiratory infections plays the major role (2). In a recent study conducted in Addis Ababa, it was reported that *Streptococcus pneumoniae* was the most prevalent (74%) pathogen identified in throat and nasopharyngeal specimens. The study also revealed the presence of other microorganisms like *Klebsiella pneumoniae* and *Staphylococcus aureus* which cause high morbidity and mortality (3). Community acquired pneumonia (CAP) is the second most common cause of adult medical admissions in Addis Ababa and the leading cause of respiratory admissions with mortality rate of 17%. *Streptococcus pneumoniae* has been reported as the most common cause of CAP in Ethiopia (4-5).

However, recent reports have described that *Streptococcus pneumoniae* has reduced its affinity for  $\beta$ -Lactam and the isolation of *Kleb. pneumoniae* strains displaying reduced susceptibility to virtually all  $\beta$ -lactams, including Cefoxitin and Penicillin-inhibitor combinations (6-8). This situation calls for search for an alternative source of antibacterial agents that would replace, those antibiotics that have been losing their effects on pneumonia causing bacteria due to reduced susceptibility of the microorganisms. The use of higher plants and preparations from them to treat infections is an aged practice and in ages past possibly the only method

available. It is also known that experimental assessments to ensure safety, efficacy, and quality of herbal drugs, crude plant materials, plant preparations and finished products have been encouraged and adequately described in the "Guideline for Assessment of Herbal Medicine" which was prepared by WHO in 1991 (9-11). Interest in plants with antimicrobial properties has revived because of the current problems associated with the use of penicillin and other antibiotics. There are a number of studies carried out to assess the value of herbal remedies including papaya preparations for the treatment of illness (11-13).

Papaya is used in traditional medicine for a variety of purposes besides its common anthelmintic, carminative, diuretic, oxytotoxic and antiinfective effects (14-15). In a recent study conducted in Jamaica, to determine the prevalence of the use of the fruit (papaya) of *Carica papaya* as topical ulcer dressings by registered nurses in Spanish Town Hospital, comments from the users of papaya suggested that topical application of the unripe fruit promoted healing and reduced odor in chronic skin ulcers (16). It has been documented in various publications that alkaloids of papaya fruits have broad spectrum antibacterial activity. Carpain (one of the major alkaloids) inhibits the bacteria that causes tuberculosis, *Mycobacterium tuberculosis* (17-18).

It is also known that fruits of Papaya (*Carica papaya*) has bacteriostatic effects against several enteropathogens such as *Bacillus subtilis*, *Bacillus cereus*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (19-21). However, there are only few reports available on the *in vitro*

effectiveness of papaya seed. The present study was therefore undertaken to evaluate the antibacterial activity of the aqueous extract of papaya seeds against three pneumonia-causing bacteria (including their clinical isolates) by the agar dilution technique. The results were compared with the minimal inhibitory concentration of tetracycline and chloramphenicol.

## MATERIALS AND METHODS

The study was conducted in the Ethiopian Health and Nutrition Research Institute, Drug Research Laboratory, in Addis Ababa between November 2000 and June 2001.

1. **Plant materials:** Papaya solution was prepared as follows: Fresh ripe papaya fruits were purchased from a local market (*Merkato*), and the seeds were collected, thoroughly cleaned.
2. with distilled water, sun dried, powdered with Mortar and Pestle, sieved with a mesh and macerated. The macerate was filtered through No.1,18.5 cm Whatman filter, cooled immediately to -20 °C in a refrigerator and lyophilized. The powder obtained was weighed, diluted with distilled water and the concentration determined. Once the concentration of the juice was determined, the rest of the juice and those obtained in subsequent repeated extractions were combined and stock solution prepared.

### Test microorganisms:

Three standard test microorganisms and three clinical isolates were included in the study. The standard microorganisms of *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were

obtained from the American Type Culture Collection (ATCC) and the clinical isolates of the same organisms were collected from the Department of Infectious and Other Diseases Research, Ethiopian Health and Nutrition Research Institute. These organisms were maintained in the laboratory on blood agar slopes at 4°C.

Culture of the test organisms were prepared following the method adopted from Matsen (27) and Leven, *et al* (28). In brief: from 4 to 6 morphologically identical colonies were picked with an inoculating loop and suspended in 5 ml of Soybean Casein Digest Broth (SCDB) and allowed to grow for 24 hours at 37°C. The turbidity of the broth culture was then equilibrated to match that of a 0.5 MacFarland standard. This provided organisms in the range of  $1 \times 10^6$  to  $5 \times 10^6$  CFU/ml. The culture was diluted to 1:100 with similar broth with the starting inoculum of  $1 \times 10^4$  CFU/ml, which is used for the test.

### Screening test for antibacterial activity:

Preliminary investigations had been carried out by using the cork borer method to test the activity of papaya extract against test microorganisms, and various inhibition zones, more than, 5mm, were observed. Following the activity screening, further antibacterial activity investigation of the extract was carried out using the agar dilution method (29). This was done by mixing the agar media with varying amounts of the papaya extract. Here, the Soybean Casein Digest Agar (SCDA) media which was used in the study was prepared as per the instructions of the manufacturer (BBL) of the dry powder and held at a temperature range of 45-50° C and mixed aseptically with the papaya seed extract (30).

The following dilutions (ml) of papaya extracts per 20ml of media were used in the study: 2 ml, 4ml, 4.5 ml, 5 ml, 7ml. This is

equivalent to papaya seed extract concentration 5.25mg/ml, 10.5mg/ml, 11.8mg/ml, 13.13mg/ml and 18.38mg/ml of media respectively. Then, after the plates were prepared in this way, they were inoculated with the test organisms. A separate agar plate without added papaya extract was also prepared in order to provide an appropriate growth control. Following inoculation, the plates were incubated at 37° C in an inverted position for 20-24 hrs in an aerobic environment.

The inhibitory effect of different concentration of papaya extract was examined by direct visual comparison of the test cultures with the control cultures. The Minimum Inhibitory Concentration (MIC) recorded as the lowest concentration of papaya extract that was capable of completely inhibiting growth of the microorganisms was then estimated. All the tests were carried out in triplicate and the results were reported as the average of these replications. Turbidity or sub culturing to quantify the rate that there is growth or inhibition was not done for this study but antibacterial effect was determined by direct visual comparison of the growth of the test cultures with the

control cultures. Quantification is suggested to determine during clinical trial and/or dosage form development.

## RESULTS

The results of the *in-vitro* susceptibility of the test organisms to the papaya extracts are given in the table. Of all the microorganisms tested, 90% were sensitive at a concentration of 4.5ml/20ml of media (11.8mg/ml of extract) and *Streptococcus pneumoniae* is sensitive at a concentration of 7 ml/20ml of media (18.38 mg/ml) and above. The results also revealed that the clinical isolate of *Streptococcus pneumoniae* is the least sensitive of the test organisms. However, compared with all the test microorganisms with the exception of *Streptococcus pneumoniae*, clinical isolate, were totally inhibited at a concentration of 11.8mg/ml of media and the MIC value was found to be 11.8 mg/ml of media. In this study, the MIC of standard drugs of Tetracycline and Chloramphenicol was also determined for the test organisms and was found to be less than 1mg/ml and 2mg/ml respectively.

Table 1. The antibacterial activity of papaya seed aqueous extract. Minimum Inhibitory Concentration (ml/20 ml of media or mg/ml of media), EHNRI, Addis Ababa, Nov 2000 - June 2001.

Bacterial species tested	2 ml	4 ml	4.5 ml	5 ml	7 ml
1. <i>Staph. aureus</i> ATCC 25923	-	-	11.8 mg/ml	+	+
2. <i>Kleb. Pneumoniae</i> ATCC 13883	-	-	11.8mg/ml	+	+
3. <i>Strepto.pneumoniae</i> ATCC 7465	-	-	11.8mg/ml	+	+
4. <i>Strepto.pneumoniae</i> (clinical isolate)	-	-	-	-	18.38mg/ml
5. <i>Kleb. pneumoniae</i> (clinical isolate)	-	-	11.8mg/ml	+	+
6. <i>Staph. aureus</i> (clinical isolate)	-	-	11.8mg/ml	+	+

N.B: + indicates inhibition of bacterial growth

## DISCUSSION

To the majority of the Ethiopian people living in the countryside traditional medicine is the only available health care system, like it is the case in other developing countries (9-12). Medicinal plants are useful not only in the traditional system of medical care at the local level but also in the production of modern medicines as source of direct therapeutic agents, as a raw materials, as taxonomic markers or as models for new synthetic products.

As modern pharmaceuticals or semi-produced products, they can help reduce imports of drugs and thus boost economic self reliance. That was why the Alma-Ata declaration recommended the use of traditional medicine in general and the uses

of medicinal plants in a more rational and scientific basis. The drugs used in traditional medicine mainly consist of cold water extracts similar to the papaya aqueous extract employed in the present study or decoction of certain plants (22, 23).

Antibacterial activity of papaya has been observed in a variety of experimental studies. It completely inhibits a variety of Gram-positive as well as Gram-negative bacteria. The papaya seed extract used in this study was found to inhibit growth and inactivated a wide range of Gram negative and Gram-positive bacteria as shown in the table. Generally, a higher concentration of the papaya extract was needed to demonstrate an inhibitory effect on clinical isolates of *Strept. pneumoniae*, indicating that *Streptococcus pneumoniae* is less

sensitive to the papaya seed extract. The present study results on the growth inhibitory activity of papaya seed against the test organisms are consistent with previous similar studies elsewhere (18-19).

In a similar study conducted in Ethiopia recently, *Staphylococcus aureus* was inhibited significantly with extracts of *Zingiber officinale*, *Echinops* spp., *Coriandrum sativum* and *Cymbopogon citratus* whereas the Gram-negative test organisms were generally less sensitive to activities of crude medicinal plants preparations (24). Similarly, other study results on growth inhibitory activity of ripe and unripe papaya fruits (epicarp, endocarp, seeds and leaves) against *Staphylococcus aureus*, *Bacillus cereus*, *E. coli*, *Pseudomonas aeruginosa* and *Shigella flexneri* indicated strong antibacterial effects on both gram-positive and gram negative bacteria. The results of the present study are in conformity with the previous results (20-21).

The results indicate values for Minimum Inhibitory Concentrations (MIC) of papaya seed extract against test organisms. At Minimum Inhibitory Concentration (MIC), 11.8 mg/ml of papaya extract, 90% of the test organisms exhibited growth inhibition. The highest MIC was recorded for clinical isolate of *Strept. pneumoniae*. In the present study, the papaya extract exhibited less antibacterial activity, in general against *Strept. pneumoniae* as compared with the other test microorganisms. Although the growth inhibitory effect on our test microorganisms would qualify the papaya seed extract for treating pneumonia caused by these test organisms, higher concentrations of the extract would exhibit complete inhibition of the test bacteria. It is, thus, quite likely that the inhibitory effect of the papaya seed

extract against those strains that had become resistant to antibiotic could be considerably enhanced in traditional treatment, if it is taken at higher concentrations.

Since papaya is a pharmacopoeial standard medicinal plant, the efficacy and safety of the plant have previously been studied. No side effects were noted, even with relatively large doses during many toxicological studies and papaya preparations have a wide range of safety margin. In a study conducted on the influence of crude aqueous extract of papaya seeds on semen profile fertility, body and organ weight response, and toxicology in male albino rats, the data revealed that reversible sterility could be induced without adverse effects on libido and toxicological profile (25-26). This indicates that papaya can be safely used in prophylaxes and treatment of common conditions related to bacterial pneumonia either as treatment supplement or alone after standardized dosage form development. The implementation of such programme would provide a relief to the health care system that has already been stressed with bacterial infections, shortage of basic antibiotics and /or microbial resistance to the commonly used antibiotics.

In conclusion, the in vitro antibacterial effectiveness of the aqueous extract of papaya seed has been well demonstrated in repeated studies and further purification and extraction of the active principle would give a true antibacterial activity comparable to standard antibiotics. Papaya seeds could therefore be used successfully for treating bacterial pneumonia because it is readily available and cheap and has no ill effects. Nevertheless, clinical trials on the effects of papaya seeds in bacterial pneumonia is

essential, before advocating large-scale therapy.

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