Annals of West University of Timişoara, ser. Biology, 2018, vol. 21 (1), pp.11-16

ANTIBACTERIAL ACTIVITY OF *CARICA PAPAYA* SEEDS ON SOME HUMAN PATHOGENS

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Received 5 January 2018; accepted 21 May 2018

ABSTRACT
The antibacterial activity of Carica papaya seeds on some human pathogens was evaluated using the disc diffusion method. The bioactive compound of the seeds was extracted using water and 95% ethanol. These were investigated for antibacterial activity on Staphylococcus aureus, Shigella dysenteriae, Salmonella typhi, Pseudomonas aeruginosa and Escherichia coli. The aqueous and ethanol extracts of the seeds were tested at 25, 50, and 100mg/ml concentrations on the bacterial isolates. Results showed that the aqueous and ethanol extracts of the seeds were effective in inhibiting all the test organisms. The ethanol extract gave a higher antibacterial activity on the test organisms than the aqueous extract. Staphylococcus aureus had the highest susceptibility to the ethanolic extract at 100mg/ml concentration with a zone of inhibition of 11.0mm. Salmonella typhi had the least susceptibility to the ethanol extract at 25mg/ml concentration with a zone of inhibition of 2.9mm. The test organisms had a higher susceptibility to the standard antibiotics chloramphenicol (12.2-13.2mm) than the seed extract. Demonstration of antibacterial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances from Carica papaya seeds for the development of new and strong antibacterial agents.
KEY WORDS: *Carica papaya, Antibacterial activity, Seeds, human pathogens, Pawpaw.*

INTRODUCTION

The search for newer sources of antibiotics has preoccupied research institutions, pharmaceutical companies and academia, since the emergence of resistance to synthetic drugs by infectious agents (Latha & Kannabiran, 2006). Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths everyday (Ahmad & Beg, 2001). The situation has been further complicated with the rapid development of multi drug resistance by microorganisms to the antimicrobial agents available (Adekunle & Adekunle, 2009). The use of local plants as primary health remedies, due to their pharmacological properties is quite common in Asia, Latin America, USA, China, Japan and Africa (Bibitha *et al.,* 2002).

The importance of herbs in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with other substances that appears to be inactive. However, these complimentary components give the plant as a

whole, a safety and efficiency much superior to that of its isolated and pure active components (Ahmad & Beg, 2001; Ianovici *et al,* 2010).

Medicinal plants are reservoirs of various metabolites and provide unlimited source of important chemicals that have diverse biological properties and represents a rich source from which antimicrobial agents can be obtained (Timothy & Idu, 2011; Ianovici *et al,* 2017).

The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Ukaegbu-Obi *et al*., 2016). Antimicrobials of plant origin effective in the treatment of infectious diseases and simultaneously mitigating many of the side effects often associated with synthetic antimicrobial agents have been discovered (Ukaegbu-Obi *et al*., 2015). Medical uses of plants range from the administration of roots, barks, stems, leaves and seeds to the use of extracts and decoction from the plants (Ukaegbu-Obi *et al*., 2015).

Carica papaya belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases (Alabi *et al.,* 2012). *Carica papaya* is a neutraceutical plant having a wide range of pharmacological activities. The whole plant has its own medicinal value. Papaya is a powerhouse of nutrients and is available throughout the year. The black seeds of the papaya are edible and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper (Alabi *et al.,* 2012).

The increasing rate of development of resistance to commonly used antibiotics has led to the search for newer, more effective, affordable and readily available sources, particularly from local medicinal plants.

This research is aimed at evaluating the antibacterial activity of the aqueous and ethanolic extracts of *Carica papaya* seeds on some pathogenic bacteria isolates *in vitro* so as to provide a guide or direction on the concentration of the seed extract active against these organisms to the populace who use them to treat various diseases caused by the bacteria isolates.

MATERIALS AND METHODS

The test organisms used are all human pathogenic organisms of clinical origin. They were obtained from National Veterinary Research Institute, Umudike, Abia state. All isolates were subcultured onto nutrient agar. Subsequent subculturing was done in plant pathology laboratory unit of National Root Crops Research Institute, Umudike for further purification. The purified bacteria were kept as stock cultures at 4°C. Biochemical analysis was carried out on each of the test organism for confirmation.

Collection and Preparation of Seed Samples. Pawpaw fruits were bought in Ndioru market in Umudike from fruit sellers. The pawpaw fruits were cut into halves. The seeds inside were removed into a clean, sterilized tray and were all sundried for four days. The dry seeds were milled with a sterilized kitchen blender and the milled powder was packed into a sterile plastic rubber bottle and kept in a cool dark place.

Preparation of Ethanolic and Cold Aqueous Seed Extract. The extraction of the seeds was carried out using ethanol and distilled water as extracting solvents. The cold maceration extraction method of Cowan (1999) was used. 5g of the ground pawpaw seeds was soaked in 100ml of sterile distilled water inside a 1 litre conical flask and sealed with foil paper (Ogunjobi & Nnadozie, 2004). The crude extracts were obtained by filtration with a clean

cheese cloth. Similarly, 5g of the ground samples were soaked in 100ml of 95% ethanol in a 1 litre conical flask and sealed with foil paper. The flasks were shaken vigorously at 30 minutes interval and left to stand for 6 hours at room temperature. The crude extracts were obtained by filtration with a cheese cloth. Both extracts were dried by evaporating in hot air oven at 60°C for 5 minutes. The methods of Akujobi *et al.* (2004) were adopted for the preparations of dilutions of crude extract for antibacterial assay. The dried solids were dissolved in DMSO to form different concentrations of 50, 75 and 100% for both aqueous and ethanolic extracts respectively.

Susceptibility Testing. The disc diffusion method of Kirby-Bauer was employed in determining the antibacterial activity of the plant extract. A loop full of each of the test organisms were introduced separately by streaking with flamed wire loop on Petri dishes containing Mueller Hinton agar which had already set and were then labeled accordingly. Sterile discs of 5mm in diameter were impregnated with different concentrations of the extracts plus the commercial antibiotic (chloramphenicol). The impregnated discs were placed in an incubator and left to dry for 2 hours. After drying, the impregnated discs were placed using sterile forceps on the streaked plates. All plates were incubated at 37°C for 24 hours. Aseptic conditions were observed throughout the experiment.

RESULTS AND DISCUSSIONS

Results obtained revealed that both the aqueous and ethanol extracts of the test plant seeds (*Carica papaya*) exhibited inhibitory effect on the test bacteria. There was no resistance from any of the bacteria. The ethanolic extract gave a higher antibacterial activity on the test organisms than the aqueous extract with zone of inhibition ranging from 2.9-11.0mm as shown in table 2. *Staphylococcus aureus* showed the highest susceptibility to the ethanolic extract at 100mg/ml concentration with a zone of inhibition of 11.0mm. *Salmonella typhi* showed the least susceptibility to the ethanolic extract with a zone of inhibition of 2.9mm. The test organisms showed a higher susceptibility to the standard antibiotics chloramphenicol than the seed extracts (12.2-13.2mm).

TABLE 1: Antibacterial activity of 95% ethanolic extract of *Carica papaya* seeds

	Zone of inhibition (mm)				
Concentration (mg/ml)	*S. aureus*	*P. aeruginosa*	*E. coli*	*S. typhi*	*S. dysenteriae*
25	3.3	3.1	3.5	2.9	3.7
50	4.9	4.5	4.6	4.2	5.2
100	11.0	8.8	9.2	6.2	7.9
Chloramphenicol	13.2	13.0	12.9	12.5	12.3

TABLE 2: Antibacterial activity of aqueous extract of *Carica papaya* seeds

	Zone of inhibition (mm)				
Concentration (mg/ml)	*S. aureus*	*P. aeruginosa*	*E. coli*	*S. typhi*	*S. dysenteriae*
25	3.0	2.9	3.3	2.6	3.5
50	4.4	4.2	4.3	4.0	5.0
100	10.6	8.5	9.0	6.2	7.6
Chloramphenicol	13.0	13.0	12.6	12.4	12.2

Results obtained showed that the aqueous and ethanolic extracts of *Carica papaya* seeds inhibited all the test organisms at all concentrations. The ethanolic extract gave a higher antibacterial activity on the test organisms than the aqueous extracts. This may be due to the better solubility of the active components of the seed in organic solvents (de Boer *et al.,* 2005). *Staphylococcus aureus* was most sensitive to the ethanolic extract at 100mg/ml (11.0mm) while *Salmonella typhi* showed the least susceptibility to the ethanol extract at a concentration of 25mg/ml (2.9mm). This is similar to the report of Peter *et al*. (2014) that investigated the aqueous, chloroform and ethanolic extract of *Carica papaya* seeds for antibacterial activity on *S. aureus, P. aeruginosa, E. coli* and *S. typhi.* It was observed that the three extracts were able to inhibit all bacteria tested but the test organisms showed a higher susceptibility to the ethanolic extracts. The chloroform extract had the least sensitivity on the test organisms.

Several other reports have shown that *Carica papaya* have significant antibacterial activity in various extracts from different tree parts (Ifesan *et al*., 2013; Nirosha & Mangalanayaki, 2013; Doughari *et al*., 2003). Also, other researchers have reported that organic extracts of the dried seed of *C. papaya*, produces microbial inhibition (Dawkins *et al.,* 2003; Emeruwa, 1982).

Also, the seed extracts showed appreciable level of potency against the commonest etiologic agent of enteric fever *Salmonella typhi*. Brooks *et al*. (2004) reported that enteric fever had mortality rate of 10-15% in developing countries. The seed extract of *Carica papaya* may as well be put into use as therapy for treating the *Salmonella* infection. Further pharmacological evaluations, toxicological studies and possible isolation of the active therapeutic ingredients will be of immense advantage in overcoming the menace of these bacterial diseases. The successful inhibition of these bacteria is a good development, especially when we consider the records of multi resistance to various conventional antibiotics by bacteria over the years.

This finding justifies the traditional uses of *Carica papaya* seed for therapeutic purposes. The plant part has records of being used as analgesic, amebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic, vermifuge and also effective against jaundice (Anibijuwon & Udeze, 2009).

CONCLUSIONS

It could be concluded that the demonstration of antibacterial activity against both gram negative and positive bacteria is an indication that the plant is a potential source for the production of drugs with a broad spectrum of activity. The result of the study also support the traditional application of the plant and suggest that the plant extract possess compounds with

antibacterial properties that can be used as antibacterial agent in novel drugs for the treatment of gastro enteritis, enteric fever, urethritis and wound infections associated with the test bacteria.

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