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Research Article

Antibacterial Properties of *Carica Papaya* Leaf and Fruit Extracts

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Abstract: *Carica papaya* has various medicinal uses ranging from promoting human health, suppressing sicknesses and diseases and also curing and preventing some diseases. The aim of this research is to determine the antibacterial properties of some parts of *C. papaya* plant (leaf, Ripe and unripe fruits). Aqueous extraction of leaf, epicarp and endocarp of unripe and ripe fruits of the plant was carried out and the extracts were diluted to 500mg/ml, then doubling dilution was carried out for 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml. Four test Bacteria used in this work were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Klebsiella pneumoniae*. The antibacterial activities of the aqueous extracts of the leaf, epicarp and endocarp of unripe and ripe fruits of *C. fistula* was determined using modified Kirby Bauer well-diffusion sensitivity testing method. The three extracts had bactericidal effect on the Gram Positive test bacteria than the Gram Negative test bacteria. The boost in medicinal herb development and sustenance should be encouraged by policy makers and philanthropists.
Keywords: *Carica papaya* medicin, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Klebsiella pneumoniae*.

INTRODUCTION

According to World Health Organization, 56% of deaths in Africa are due to infectious diseases, maternal, perinatal or nutritional conditions (WHO, 2015). Some gram positive and gram negative pathogenic bacteria, such as *Escherichia coli, Vibrio cholerea, Klebsiella sp, Slamonella sp, Shigella sp, Staphylococcus aureus* are commonly incriminated in bacterial infections. Public health significance of antibiotic resistance by these microbes is growing at an alarming rate globally (Mulligen, *et al.*, 1993). Due to continuous use, misuse and abuse of antibiotics, microorganisms have grown more resistant. As a result, an alternative source of therapy draws the world's attention. One major alternative is the use of herbs.

The use of herbs to improve and maintain health dates as far back as the days of the early man, when man used raw or cooked herbs across the world. However, in recent years better approaches towards medicinal use of herbs have increased the precision of some specific applications of these plants which were previously unknown (Kafaru, 1994). In 1993 according to Namita and Mukesh (2012), 80% of the world's population depends on traditional medicine for treatments of infectious diseases.

Some of these plants have antimicrobial properties and are the back bone of many powerful drugs widely distributed globally, while others have not fully been exploited and as a result are mostly consumed unprocessed (Cowan, 1999). Medicinal plants have diverse uses, some of which are for the reduction of swellings and inflammation on the skin, subsiding of stomach discomfort and cramps, reducing blood sugar levels, prevention of cancer, and used as antimicrobial agents (Cowan, 1999; Kooti *et al.*, 2016). Examples of such plants are *Cassia fistula*, Garlic (*Allium sativum*), Dandelion, Moringa (*Moringa oleifera*), Thyme (*Thymus vulgaris*) and Pawpaw (*Carica papaya*).

The Pawpaw plant produces a cerise-orange colored fruit with an attractive fragrance, edible, delicious, juicy and also very healthy. Almost every part of the pawpaw plant has various uses. The fruit has

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an impressive demand worldwide which means farmers who grow this plant sell and make money from it, which gives it a commercial or economic importance. The ripe fruit of the *C. papaya* is delicious, juicy and nutritious giving it a proper ingredient for a fruit salad. The unripe fruit which is rich in papain can also be eaten raw or cooked and served in several dishes; in countries like Indonesia the flower buds are used for making papaya flower vegetable dish. The ripe pawpaw fruit is also used for making of sweets and preservatives. All these make up the culinary uses of the *C. papaya*.

In addition, *C. papaya* has various medicinal uses ranging from promoting human health, suppressing sicknesses and diseases and also curing and preventing some diseases. Due to richness in vitamin C, antioxidants, papain and other substances, the papaya plant is known to prevent cancer, boost immunity, protect against arthritis, improve digestion, hair growth enhancement, lowers cholesterol, prevents signs of ageing, contains antimalarial and also antimicrobial properties(Sengupta, 2019) . Researchers over the years have proven the *C. papaya* leaf is a high potential source of secondary metabolites containing high antifungal properties against *Fusarium* spp, *Colletotrichum gleosporioides*, *Microsporum canis* and other species of yeast like *Candida* spp (Cha´vez-Quintal et.al. 2011). In bacteria, it was found to be active against *Staphylococcus aureus* which is incriminated in boils, breast abscess and most surgical infections (Anibijuwan and Udeze, 2009). The aim of this research is to determine the antibacterial properties of some parts of *C. papaya* plant (leaf, Ripe and unripe fruits).

Materials and Methods

Plant Collection

Samples were collected from randomly selected species of *C. papaya* (leaf, ripe and unripe fruits), thoroughly washed and the fruits were cleaned with 45% ethanol to reduce the microbial load on the back of the fruits. While the leaves were sliced, the fruits (unripe and ripe) were peeled, sliced and separately dried as epicarp and endocarp. Each of them were dried separately at 40^{o}C in a hot air oven.

Extraction process

Aqueous extraction (Hot water) was carried out for all the extracts. Dried papaya parts were finely pounded and homogenized in hot water for one hour, filtered using WhatMan No. 1 filter paper and steam dried. Extracts were weighed and labeled. The extracts we're diluted to 500mg/ml then doubling dilution was carried out for 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml.

Test Bacteria

Four test Bacteria used in this work were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Klebsiella pneumoniae*. They were bacterial isolates of livestock preserved in agar slants gotten from the Microbiology Laboratory of Federal College of Animal Health and Production Technology Vom.

Antibacterial screening by zone of inhibition

The antibacterial activities of the aqueous extracts of the leaf, ripe and unripe endocarps and the epicarps of both the ripe and unripe *C. papaya* plant was determined using modified Kirby Bauer well-diffusion sensitivity testing method. Overnight broth culture of each of the test bacteria was adjusted to 0.5 McFarland's turbidity standard using sterile normal saline. Thereafter, 0.2ml of the adjusted broth culture were added to 20mls of molten Nutrient Agar before aseptically pouring into a petri dish. After solidifying, seven holes with diameters of 6mm were made on the media using a sterile cork-borer and labeled appropriately. Five holes had the concentrations of the respective extracts, while the other two had the positive and negative controls respectively. Ampiclox of 50mg/ml concentration was used as the positive control while sterile distilled water was used as the negative control. Incubation was done at 37°C for 24 hours. The diameter zone of inhibition was measured using a transparent plastic ruler in millimeter.

Minimum inhibitory concentration (MIC)

The MIC of each of the extracts was determined using the tube serial dilution method. Extracts concentrations of 500, 250, 125, 62.5, 31.25, mg/ml were used. An overnight broth culture of the test bacteria was adjusted to 0.5 McFarland turbidity standard (10^{6} CFUs/ml) and 0.2 ml of the cell suspension were added to each of the tubes containing the extracts. The tubes were incubated aerobically at 37°C for 24 hours. The MIC was defined as the lowest extract concentration that inhibited the growth of the test organism as indicated by absence of visible turbidity in the tube compared with the control tubes.

Result

All the extracts at different concentrations had effect on both gram positive and gram negative test bacteria. The antimicrobial activity results were shown in Tables 1 to 5 for leaf, unripe epicarp, ripe epicarp, unripe endocarp and ripe endocarp respectively.

It was observed from the tables below that at 500mg/ml, *Staphylococcus aureus* was more susceptible to *C. papaya* leaf with a zone of inhibition of 29mm while *E. coli* with a zone of inhibition of 15mm had the lowest susceptibility. The unripe epicarp extract was more active against *Bacillus subtilis* and wasn't active at all against *S. aureus* and *K. pneumoniae*. The ripe epicarp extract was also more active against *B. subtillis* and had no effect against *E. coli*. The extract of the unripe endocarp was more active against *B. subtillis* and not active against *K. pneumoniae* while the extract of the ripe endocarp was also active against *B. subtillis* with *E. coli* having the least susceptibility.

Table 1. Antibacterial activity of *C. papaya* leaf extracts

Bacteria	**Zone of inhibition (mm)**						
	500mg/ml	**250mg/ml**	**125mg/ml**	**62.5mg/ml**	**31.25mg/ml**	**Positive control**	**Negative control**
Escherichia coli	15	12	10	6	0	48	0
Klebsiella pneumoniae	17	15	10	8	0	17	0
Bacillus subtillis	17	12	7	7	6	22	0
Staphylococcus aureus	29	25	20	15	6	56	0

Table 2. Antibacterial activity of unripe *C. papaya* epicarp extracts

Bacteria	**Zone of inhibition (mm)**						
	500mg/ml	**250mg/ml**	**125mg/ml**	**62.5mg/ml**	**31.25mg/ml**	**Positive control**	**Negative control**
Escherichia coli	18	16	9	7	6	28	0
Klebsiella pneumoniae	7	7	6	6	0	15	0
Bacillus subtillis	24	21	20	16	11	50	0
Staphylococcus aureus	10	8	7	6	6	40	0

Table 3. Antibacterial activity of ripe *C. papaya* epicarp extracts

Bacteria	**Zone of inhibition (mm)**						
	500mg/ml	**250mg/ml**	**125mg/ml**	**62.5mg/ml**	**31.25mg/ml**	**Positive control**	**Negative control**
Escherichia coli	8	7	7	6	0	70	0
Klebsiella pneumoniae	12	9	7	7	0	15	0
Bacillus subtillis	25	21	13	12	6	30	0
Staphylococcus aureus	24	18	12	10	6	59	0

Table 4. Antibacterial activity of unripe *C. papaya* endocarp extracts

Organism	**Zone of inhibition (mm)**						
	500mg/ml	**250mg/ml**	**125mg/ml**	**62.5mg/ml**	**31.25mg/ml**	**Positive control**	**Negative control**
Escherichia coli	10	8	7	6	0	64	0
Klebsiella pneumoniae	9	7	6	6	0	23	0
Bacillus subtillis	23	19	16	11	10	29	0
Staphylococcus aureus	12	8	7	7	6	55	0

Table 5. Antibacterial activity of ripe *C. papaya* endocarp extracts

Organism	**Zone of inhibition (mm)**						
	500mg/ml	**250mg/ml**	**125mg/ml**	**62.5mg/ml**	**31.25mg/ml**	**Positive control**	**Negative control**
Escherichia coli	17	15	10	9	0	53	0
Klebsiella pneumoniae	23	14	12	8	0	29	0
Bacillus subtillis	28	24	23	18	17	29	0
Staphylococcus aureus	20	18	11	9	0	50	0

The mean result of MIC for all the extracts was 125 mg/ml.

Discussion

The results of this study shows that the extracts of the *C. papaya* plant have bacteriostatic effects on all test bacteria used, which means that some of these extracts can be used to suppress infections caused by or related to the test organisms. This is in line with the study carried out by Akujobi *et al.*, 2010 where it was discovered that pawpaw fruits were used to treat wounds and some fevers in some rural areas in Nigeria. The epicarp, endocarp and leaf extracts showed antibacterial activities on the test bacteria. However, these activities were more against Gram positive test bacteria than the Gram negative ones. This is in line with the report of Akujobi *et al.,* 2010 where papaya extract had bacteriostatic effect on *E. coli* but a bactericidal effect on *S. aureus*. Ripe endocarp had the highest effect on the Gram positive test bacteria while the unripe endocarp had the least effect on them. The fact that the extracts had effect on both Gram positive and Gram negative bacteria could be indicative of their broad spectrum potency (Anibijuwon and Udeze, 2009). The use of only aqueous method of extraction may have reduced the potency of these extracts. It is encouraged that ethanolic extraction should be carried out in the future since it is known that it is a better solvent of extraction than water.

Conclusion

It is well known that any use of antimicrobials however appropriate and justified, contributes to the development of resistance, but widespread misuse makes the situation worse. The threat of antimicrobial resistance (AMR) is growing at an alarming rate, a situation perhaps aggravated in developing countries due to gross abuse in the use of antimicrobials. As a result, scientific studies should shift to affordable alternative such as *C. papaya* and other medicinal herbs.

It is recommended that pharmaceutical organizations, research-funding organizations, governments, Non-governmental organizations (NGOs) and other well-meaning individuals should boost research in this field in other to curb the increase in the spread of antimicrobial resistant pathogens.

Conflicts of Interest

The authors declare that they have no conflicts of interest in publishing this manuscript.

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