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Antibacterial properties of the ripe endocarp of carica papaya (pawpaw) fruit on staphylococcus aureus

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Abstract

The broth tube dilution and agar disc diffusion test methods were used to assess antibacterial properties of the extracts of ripe Carica papaya fruit on Staphylococcus aureus. The results of the antibacterial tests show that the extracts were antibiotic on the bacteria. I*nhibition zones were produced by the ethanol, methanol and ethanol-water extracts. The test results also indicate that ethanol and methanol extracts produced the MIC result of 0.013g/mL while ethanol -water showed the MIC of 0.025g/mL. However, the result of the T-test of scores shows no significant difference between the extracts (0.027<0.05). Therefore, consumption of ripe endocarp of C. papaya is recommended for its curative benefits against S. aureus*.

Keywords: Antibacterial, properties, ripe endocarp, carica papaya (pawpaw) fruit, staphylococcus aureus.

Introduction

World Health Organization states that antibiotic resistance is one of the biggest threats to global health, food security and development today[1]. Antimicrobial resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them, the germs are not killed and continue to grow.

This is partly because plant metabolites and bio-active compounds from chemical synthesis have found their efficacy to be dwindling despite being used for several decades due to upsurge in drug resistance[2].

The gram positive bacteria are more of concern especially pneumococci, enterococci and staphylococci with *S. aureus* of more concern due to its intrinsic virulence, its ability to cause a diverse array of life threatening infections and its capacity to adapt to different environmental conditions. *S. aureus* has been implicated in a wide range of infections on the skin, soft tissue, respiratory tract, bone joints, endovascular system and wound infections.

It is also the leading cause of nosocomial infections and as more patients are treated outside the hospital setting, the danger is more pronounced. This, in addition to its antibiotic resistance, is a serious cause for concern[3,4].

Over the years, numerous investigations into the inhibition of microorganisms by spices, herbs, fruits and vegetables consumed as natural foods have been reported, with natural products as the reservoir of antimicrobial compounds. Natural products are known to provide the metabolites needed for medicinal and health products. Many researchers have found solutions to diverse challenges from natural products, metabolites and extracts[5,6].

One of the natural foods widely consumed its nutritional value as well as its medicinal benefits are papaya, a large herbaceous plant often mistaken for a tree grown in the tropics and sub-tropics. The fruit is referred to as "the common man's fruit" and as a "quasi drug" owing to its medicinal efficacy. Papaya has been reported to serve as remedies and supplements using different parts of the plant[7-9].

The need to investigate the antimicrobial ability of this widely consumed fruit in view of serving a highly desirable need for controlling *Staphylococcus aureus* is the basis for this research.

Materials and Methods

Overview of Experimental Procedure: To assess comprehensively the antibacterial properties of the ripe endocarp of *C. papaya* two tests were carried out: inhibition and bactericidal tests. Inhibition and bactericidal potentials were assessed using agar dilution method. This method involves the incorporation of different concentrations of the antimicrobial substance into a nutrient medium followed by the application of a standard number of cells to the surface of agar plates[10].

Five different extracting solvents were used: ethanol, methanol, methylated spirit, ethanol: water (50:50) and water (aqueous). The phytochemical screening was carried out at the Chemistry Research Laboratory of the Taraba State University, Jalingo, Nigeria while the antibacterial tests were undertaken at the microbiology laboratory of the Federal Medical Centre, Jalingo,

Taraba State, Nigeria. The following procedure was followed: (i) Preparation of extracts, (ii) Preparation of inoculums, (iii) Serial dilution of extracts, (iv) Inhibitory tests and (v) Bactericidal tests.

Preparation of Extracts: Ripe endocarp of pawpaw was bought in Jalingo market, thoroughly washed and shade dried for ten days. The dried weight was measured (2g) into each of the five different extracting solvents (20mL) to yield an initial concentration of 1:10 g/mL each[11]. The containers were sealed and left to stand for four days[12] after which they were centrifuged for 10 minutes at 4000 rpm at 25˚C. The mixture was then filtered and the supernatant collected and stored at 4˚C until further use[13].

Preparation of Inoculum: 5 ml of sterile saline water was poured into a measuring cylinder[14;15] into which pure isolates of *Staphylococcus aureus* from the microbiology laboratory of Federal Medical Centre Jalingo (Lab. No. 40846 GOPD) was added and mixed thoroughly. From this mixture, 0.2mL was pipetted into a measuring cylinder containing 40mL of sterile saline water and also mixed. The needed concentration (0.5 McFarland Standard Solution) was extracted from here for inoculation on agar plates for antibacterial tests[16].

Serial Dilution of Extracts: The serial dilution of the different extracts was carried out as outlined by Rollins and Joseph[17]. The dilution was carried out as follows: 2mL of extract was put into the first test tube of six (6) labelled tubes; 1mL of sterile saline water was put into the remaining tubes (2-6). Using a sterile syringe, 1mL of extract was removed from test tube 1 and transferred to test tube 2 and mixed. Subsequently, 1mL was transferred from test tube 2 to 3 and so on up to test tube 5. Test tube 6 did not receive any extract. (Control) Subsequently, 1 mL of liquid was removed from test tube 5 and discarded. This ensured that the concentration of extract in one tube was one-half the concentration in that of the preceding tube (from test tube 1 to 2, from 2 to 3 in that order). The volume however, remained the same (1mL in each tube). It was from these tubes of varied concentrations that extracts were matched with the standard inoculums of *Staphylococcus aureus*.

Inhibitory Test Using Agar Wells: Agar plates prepared with nutrient agar and wells of 5mm made on the plates in triangular positions 1.5 inches apart. The plates were then labelled (1-6) with a total of three replicates per concentration for each extract[11]. The plates were inoculated using a sterile loop with streaking over the entire surface to ensure even distribution of inoculums and allowed to dry. 50mL aliquots of each test extract were dispensed into the corresponding numbered plate and allowed to dry.

The plates were then covered and incubated at 37˚C for 24 hours after which the zones of inhibition were measured with a meter rule (mm) with the measurement starting point from the edge of the well[11,13].

Determination of the Minimum Inhibitory Concentration (MIC): The MIC is determined by assessing the growth of *S. aureus* after incubation and the lowest concentration of the different ripe extracts of *C. papaya* that inhibited the growth of the bacteria was noted[17].

Bactericidal Test (To determine MBC): Agar plates were inoculated with the standard inoculums of *S. aureus* on numbered plates as described above. The plates were allowed to dry after which the various concentrations of extracts were dropped on the inoculated plates. The least concentration of extract showing the absence of bacterial growth was to be taken as the minimum bactericidal concentration[16].

Statistical Analysis: The analysis of variance (ANOVA) to determine significance of differences of zones of inhibition produced by the different extracting solvents was also carried out at 5% degree of freedom (95 % confidence level).

Results and Discussion

Results are mentions in following Tables.

Table-1: Determination of Inhibition by the Ethanolic Extract.

Extract Conc (g/mL)	Zone of Inhibition (Diameter)	
	Conc. Ratio	(mm)
0.100	1/1	6
0.050	½	5
0.025	¼	2
0.013	1/8	1
0.006	1/16	0
0.000	1/32	0

Table-2: Determination of Inhibition by the Methanolic Extract.

Extract Conc (g/mL)	Zone of Inhibition (Diameter)	
	Conc. Ratio	(mm)
0.100	1/1	5
0.050	½	2
0.025	¼	1
0.013	1/8	1
0.006	1/16	0
0.000	1/32	0

Table-3: Determination of Inhibition by the Methylated Spirit Extract.

Number	Extract Conc (g/mL)	Zone of Inhibition (Diameter)	
		Conc. Ratio	(mm)
1.	0.100	1/1	0
2.	0.050	½	0
3.	0.025	¼	0
4.	0.013	1/8	0
5.	0.006	1/16	0
6.	0.000	1/32	0

Table-4: Determination of Inhibition by the Ethanol-Water Extract.

Number	Extract	Zone of Inhibition (Diameter)	
	Conc (g/mL)	Conc. Ratio	(mm)
1.	0.100	1/1	2
2.	0.050	½	1
3.	0.025	¼	1
4.	0.013	1/8	0
5.	0.006	1/16	0
6.	0.000	1/32	0

Table-5: Determination of Inhibition by the Water (Aqueous) Extract.

Number	Extract	Zone of Inhibition (Diameter)	
	Conc (g/mL)	Conc. Ratio	(mm)
1.	0.100	1/1	0
2.	0.050	½	0
3.	0.025	¼	0
4.	0.013	1/8	0
5.	0.006	1/16	0
6.	0.000	1/32	0

Table-6: Bactericidal test by the ethanolic extract.

Number S/n	Extract	Bacterial growth	
	Conc (g/mL)	Conc. Ratio	Present
1.	0.100	1/1	✓
2.	0.050	½	✓
3.	0.025	¼	✓
4.	0.013	1/8	✓
5.	0.006	1/16	✓
6.	0.000	1/32	✓

Table-7: Bactericidal test by the methanolic extract.

Number	Extract	Bacterial growth	
S/n	Conc (g/mL)	Conc. Ratio	Present
1.	0.100	1/1	✓
2.	0.050	½	✓
3.	0.025	¼	✓
4.	0.013	1/8	✓
5.	0.006	1/16	✓
6.	0.000	1/32	✓

Table-8: Bactericidal test by the methylated spirit extract.

Number	Extract	Bacterial growth	
	Conc (g/mL)	Conc. Ratio	Present
1.	0.100	1/1	✓
2.	0.050	½	✓
3.	0.025	¼	✓
4.	0.013	1/8	✓
5.	0.006	1/16	✓
6.	0.000	1/32	✓

Table-9: Bactericidal test by the ethanol-water extract.

Number	Extract	Bacterial growth	
S/n	Conc (g/mL)	Conc. Ratio	Present
1.	0.100	1/1	✓
2.	0.050	½	✓
3.	0.025	¼	✓
4.	0.013	1/8	✓
5.	0.006	1/16	✓
6.	0.000	1/32	✓

Table-10: Bactericidal test by the aqueous (water) extract.

Number	Extract	Bacterial growth	
S/n	Conc (g/mL)	Conc. Ratio	Present
1.	0.100	1/1	✓
2.	0.050	½	✓
3.	0.025	¼	✓
4.	0.013	1/8	✓
5.	0.006	1/16	✓
6.	0.000	1/32	✓

Discussion: The presence of inhibition zones is an indication of antimicrobial activity[18-20]. It can therefore, be stated that the presence of inhibition zones in the investigation involving extracts of ripe endocarp of *Carica papaya* on *Staphylococcus aureus* can rightly be regarded as a confirmation of antibacterial activity by the extracts. Inhibition zones were produced by the ethanol, methanol and ethanol-water extracts. The test results also indicate that ethanol and methanol extracts produced the MIC result of 0.013g/mL while ethanol -water showed the MIC of 0.025g/mL.

However, both water (aqueous) and methylated spirit did not show antibacterial activity, thereby highlighting their deficiencies as extractive solvents[21,22]. Methanol and ethanol have been extensively used for extraction of antioxidant substances from plants and plant based foods[11,13,23,24]. As this experiment has shown, the antioxidant capacities of extracts is directly related to solvent employed due to antioxidant potential[25,26].

The result of the antibacterial finding of this study agrees with many investigations on the antimicrobial property of extracts of different parts of *Carica papaya*[19,27,28]. It also agrees with other investigations into the antibacterial property of *Carica papaya* extracts against *Staphylococcus aureus* who have reported antibacterial activity by the extracts[18,22].

Given that *S. aureus* is a common wound organism and a common post surgery injury infection, this finding agrees with several investigations into the antimicrobial effects of *C. papaya* extracts on wound organisms[20,29]. The relevance of this finding in this regard cannot therefore, be overemphasized.

Given the above demonstration of antimicrobial activity, the presence of certain phytochemicals isolated from the phytochemical screening could account for antimicrobial activity. For instance, flavonoids are referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogen. They show anti-allergic, anti-inflammatory, antimicrobial and anti-cancers activities[30,31]. Similarly, tannins are best known to possess general antimicrobial and antioxidant activities while saponins are also known as bioactive antibacterial agents of plants[32,33].

Bactericidal tests carried out indicated that none of the extract was bactericidal on the test organism. This could be due to the low concentration of extracts (0.1g/mL for the highest concentration) or possibly a limitation of the extracting method used or more likely its limitation as an antimicrobial agent.

Conclusion

The results from the different tests carried out to assess the antibacterial properties of the ripe endocarp of *Carica papaya* on *Staphylococcus aureus* show that *C. papaya* inhibits *S. aureus* with the ethanol extract showing the highest efficacy. However statistical analysis shows that there is no significant difference in the inhibition zones generated by the different extractive solvents. This is in agreement with the work of many researches on the antimicrobial ability of natural foods and specifically on *Caricapapaya*. However, the extracts did not show any bactericidal activity on the bacteria- possibly an indication of its limitation as an antimicrobial substance.

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