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Antimicrobial Activity of Methanol Crude Extract and Alkaloid Fraction of *Carica Papaya* Leaves on Methicillin Resistant *Staphylococcus aureus*

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Abstract

Therapeutic plant parts since time immemorial have been used by man for the treatment of numerous diseases. For this reason, this study was carried out to determine the antibacterial activity of the crude extract as well as the alkaloid fraction of *carica papaya* leaves against Methicillin Resistant *Staphylococcus aureus* (MRSA). Phytochemical screening was done following standard procedures and the minimum inhibitory concentration (MIC) and susceptibility of the samples of the organism (MRSA) to various concentration of the methanol (crude) extract and the alkaloid fraction of *Carica papaya* was determined using the agar well diffusion technique. Phytochemical screening of the leaves reveals the presence of tannins, alkaloids, proteins, reducing sugar and phenols. The leaf extract and Alkaloid fraction demonstrated mild to moderate antimicrobial activity against the microorganism at different concentrations with high zones of inhibition $11\pm 0.000\text{mm}$, $7\pm 1.414\text{mm}$, $4\pm 0.000\text{mm}$, $4\pm 0.000\text{mm}$ respectively for the alkaloid fraction although lower concentrations of the extracts and fraction showed no observable activity. Generally, the samples of MRSA used appeared to be more susceptible to the alkaloid fraction than the crude extract of the *Carica papaya*. The alkaloid fraction of the plant's leaves had comparable and even higher activity to that of Oxacillin used. The finding from this study may be of interest and the need for further investigation in terms of toxicological and antimicrobial studies with the sole purpose of using plant in novel drug design and development.

Keywords: *Carica papaya*, MRSA, Crude extract, Alkaloid fraction, Antimicrobial

Introduction

Plants are biological species, that are ubiquitous. They have found various applications in everyday life and have been great sources of novel drugs and medicinal products likewise food materials. It is important to note that man has utilized this great treasure in enhancing his quality of life. In the area of medicine, man has been able to compound drugs for various ailments using plants, this is of great advantage since they are cheaper and safer that is, less toxic compared to synthetic drugs.

Advances in science has made it possible to isolate special constituents of plants which are considered to be active against a host of infectious diseases. These actives are called secondary metabolites. Due to the increasing toxicity reports concerning synthetic drugs, the need for research on non-synthetic drugs such as drugs obtained directly from plants and animal cannot be overemphasized.

According to the World Health Organization, Herbal medicines is the term given to finished labelled medicinal products that contain active ingredients aerial or underground part of plants and other plant materials or a combo thereof whether in a crude state or as plant preparations (WHO 1978). Examples of antimicrobial agents include antiseptics, germicides, antibiotics, antifungal and other anti-infectives. A distinction however must be made between antiseptics and chemotherapeutic agents such as antibiotics.

Antibiotics are said to be substances that are produced by microorganisms, which selectively suppress the growth or kill other microorganisms at very low concentration. This definition excludes other natural substances which also inhibit microorganism but are produced by higher forms for example antibodies, or even those produced by microbes but are needed at high concentrations for example ethanol, lactic acid, H₂O₂ (Tripathi 2014). With the study of active ingredients present in herbal medicines and formulations, new drug products can be discovered which can thereafter be subjected to clinical trials and research. Chemotherapy makes reference to the treatment of diseases with chemicals, current usage of the term implies the treatment of infectious diseases and cancers and when making reference to the infectious disease, antimicrobial chemotherapy is used. Antimicrobial chemotherapy is the clinical application of antimicrobial agents to treat infectious disease. Antimicrobial chemotherapy can be used either for prophylaxis (prevention) or treatment (cure) of disease caused by bacterial, fungi, viruses, protozoa or helminthes (Dennis *et al.*, 2014).

Plants are seen as important sources of chemotherapeutic agents besides microbes, animals, minerals and other synthetic sources. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune-compromised patients in developing countries (Al-Bari *et al.*, 2006). The increasing prevalence of multi-drug resistant samples of bacteria and the recent appearance of samples with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (Rojas *et al.*, 2006, Zy. *et al* 2015).

The MRSA is a bacterium that causes infection in different body parts and due to its resistance to commonly used antibiotics, it is difficult to treat. The signs and symptoms are dependent on the area of infection, MRSA can cause mild infections of the lungs, urinary tract, blood stream and surgical wounds (Abdelkarim, 2010).

MRSA is a global public health problem. The aim of this study is to determine the potential role of use of plant products such as the leaves of *Carica papaya* as alternate treatment of infections caused by MRSA. *Carica papaya* products have a wide range of application, for example, traditionally the leaves are used in the treatment of urinary tract infections. The milky juice of *Carica papaya* when extracted and dried, is used as chewing gum, medication for digestion problems, toothpaste and meat tenderizers. It has been used to treat digestive problems and intestinal worms as well as warts, sinusitis, eczema, cutaneous tubercles and hardness of the skin. Green fruits are used to treat high blood pressure, roundworm infection, dyspepsia, constipation, amenorrhea, skin disease, general debility and genito-urinary disorders (Sandhya *et al.*, 2015). The antimicrobial properties of the *Carica papaya* plant thus informed the need for this study. The significance and benefit of this study is the need to ascertain the effect of the methanol extract of *Carica papaya L.* on methicillin resistant *Staphylococcus aureus*.

Materials and Methods

The materials, equipment and reagents used during the course of this research work include: Incubator, Bijou bottles, conical flasks, weighing balance, spatula, distilled water, filter paper, funnel, a pair of forceps, Petri dishes, Binocular microscope, Rotary evaporator (PID Temperature controller super fit digital bath), Ethanol, Methanol, Ammonia, Safranin, Lugol iodine, Crystal violet, Acetic acid, Muller Hinton agar, nutrient broth, *Carica papaya* leaves, Cloxacillin, sterile papers, nutrient agar (TM Media), sterile swab sticks, mannitol salt agar (TM Media, Titan Biotech Ltd, India), water bath shaker (Labtech AVF 500), meter rule, sterile grinding machine, salvon (comprises of 2.8% n-propyl alcohol, 0.3g chlorhexidine gluconate and 3.0 cetrimide).

Preparation of bacterial isolate from the nostrils of various human subjects

Twenty Sterile swab sticks were used to swab the nostrils of various human subjects after a duly signed informed consent form was obtained. Prepared mannitol salt agar was poured into twenty petri dishes and allowed to solidify,



thereafter each swab stick was used to streak each agar plate and all the culture plates were incubated at a temperature of 37⁰c for 24 hours and the growth patterns and colors of the organisms were observed for each culture plate. A colony for each of the petri-dishes (culture plate) showing growth of *Staphylococcus aureus* was picked using a sterile inoculating loop into a McCartney bottle containing slants of nutrient agar, maintaining aseptic conditions and stored in a refrigerator for further use.

Preparation of subcultures

The agar plates were prepared using the manufacturers specification by dissolving Muller Hinton agar in distilled water in the ratio 38g to 1000ml of distilled water in a beaker. Twenty milliliters (20ml) each of the dissolved Muller Hinton agar was poured into a sterile beaker and autoclaved at a temperature of 121⁰c for 15 minutes. This was allowed to stand in order to cool before being poured into sterile petri-dishes and allowed to solidify. The test organisms were streaked on the agar plates and incubated in an incubator at 37⁰c for 24 hours.

Identification testing for *Staphylococcus aureus*

Gram staining technique was also employed to confirm if it was gram positive and the organism was further cultured in mannitol agar to determine if the organism can grow and ferment the agar from pink to yellow color, the fermentation of the agar indicates that the test organism is *Staphylococcus aureus*.

Collection of plant materials

The leaves of *Carica papaya* Linn. were collected in Abraka, Ethiope East Local Government, Delta State, South-South Nigeria. Identification and authentication of specimen was done in the Department of Pharmacognosy and Traditional Medicine of the Faculty of Pharmacy, Delta State University, Abraka, Delta State, Nigeria.

Preparation of plant material for extraction

The fresh leaves obtained were properly washed with distilled water, then allowed to air-dry under room temperature between 35-37 °C for two weeks. Thereafter, the leaves were ground

into coarse powder using a sterile grinding machine. The powdered material was weighed and the measured mass was 800grams. The extraction process used was the cold maceration, methanol and distilled water were the solvents used in the extraction process.

Extraction of the plant material by cold maceration.

The powdered plant material was extracted using cold maceration which operates on the principle of absorption. Four hundred grams (400g) of the plant material (powdered leaves sample) was weighed with a weighing balance and poured into a sterile bucket, thereafter 2 liters of methanol was measured and transferred into it. Distilled water (0.5 liters) was measured using the measuring cylinder and poured into the resulting mixture. Thus 80% methanol was used. The mixture was stirred properly and covered. It was allowed to stand for 7 days at room temperature. The mixture was agitated regularly during this period. After completion of 7 days, the mixture was filtered and concentrated using a rotary evaporator till a paste-like extract was obtained. The paste-like crude extract was poured into porcelain dishes and stored at a temperature of 4⁰c.

Phytochemical Screening

Test for alkaloids and tannins was done as described by Ciulci (1994), flavonoids by Sofowora (1993). Test for terpenoids, reducing sugars saponins by Brain and Turner (1975). Test for phenol and quinones by Harbone (1999). While test for proteins was done as described by Pallavi *et al.* (2018).

Serial dilution of cloxacillin

10 mg of Cloxacillin powder was weighed using an analytical balance, this was dissolved in 10 ml of sterile water in a test tube. 10-fold serial dilution was done in 4 test tubes containing 9 ml of sterile water and a 5th test tube containing 5 ml of sterile water. The dilution formula $C_1V_1=C_2V_2$ was used for the serial dilution.

Preparation of cloxacillin susceptibility disks

10 small sized circular-shaped filter papers were sterilized in a beaker covered using an autoclave at a temperature of 121⁰c for 15minutes. The 5 μ g

concentration of the cloxacillin solution (5ml) obtained after the serial dilution was poured into the beakers containing the sterile papers before they were used.

Determination of methicillin-resistant *Staphylococcus aureus* using the cloxacillin susceptibility disks

Eight petri dishes were placed on an already disinfected working table. The petri dishes were labelled according to the sample of the staphylococcus aureus to be used. 2.56g of nutrient agar was weighed and dissolved in 160 ml of distilled water in a beaker and properly stirred. This was autoclaved for 15 minutes at a temperature of 121°C. The nutrient agar was allowed to cool to a temperature of 45°C before pouring the 20 ml of the agar using a sterile calibrated test tube. The agar was allowed to solidify before inoculating the agar with *staphylococcus aureus* isolates which were collected from freshly prepared overnight broth cultures, using sterile swab sticks.

With the use of a sterile forceps, the two cloxacillin disks were picked and placed in each culture plate in alternate positions. The culture plate was incubated for 24 hours at 37°C thereafter, the result was observed and recorded.

Serial dilution of *Carica papaya* leaves extract

2 g of the leaves extract of *Carica papaya* was weighed and placed in a sterile petri dish before dissolving it in 4ml of sterile water. The resulting mixture was transferred into a sterile test tube. 2ml of sterile water was measured and transferred into 5 different test tubes on the test tube rack and two-fold serial dilution was carried out to obtain the concentrations: 500mg, 250mg, 125mg, 62.5mg, 31.25mg and 15.625mg. The dilution formula $C_1V_1=C_2V_2$ was used for the serial dilution.

Determination of inhibitory activity of *Carica papaya* crude extract

The sensitivity of selected MRSA isolates to methanol extract of *Carica papaya* was determined using agar diffusion method. Seven (7) petri dishes were placed on working table, a marker pen was used to divide each petri dish into 3 halves with each petri dish appropriately

labelled with a masking tape. Twenty milliliters (20 ml) of Muller-Hinton agar was measured and poured into each petri dish before allowing to cool and solidify.

Sterile swab sticks were used to inoculate the petri dishes with overnight broth culture of the MRSA respectively. A 6 mm cork borer was used to bore holes in the solidified agar on in each culture plate, with the aid of a sterile pipette, few drops of each concentration of the extract were added to their respective holes in the culture plates. All petri dishes were packed into an incubator to allow to incubate at 37°C for 24 hours. The zones of inhibition were observed and recorded after 24 hours of incubation.

Extraction of alkaloid fraction from *Carica papaya* leaves

Forty grams (40 g) of powdered sample of *Carica papaya* leaves was weighed into a beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated in a water bath shaker, to one-quarter of the original volume, concentrated ammonia of 100ml was measured and poured into the extract till the precipitation was complete immediately after filtration, after few hours of mixture sedimentation, the supernatant was discarded and the precipitates was washed with dilute ammonia and then filtered. The residue which is the alkaloid was dried and weighed.

Serial dilution of alkaloid fraction

One gram (1g) of the alkaloid fraction was weighed and dissolved in 2ml of sterile water in a test tube. One milliliters (1ml) of sterile water was measured and poured into 6 different sterile test tubes. Two-fold serial dilution was carried out with the test tubes containing the sterile water to obtain the following concentrations; 500mg, 250mg, 125mg, 62.5mg, 31.25mg, 15.625mg and 7.8125mg. The test tubes were covered with aluminum foils they were ready for use.

Determination of inhibitory activity of the alkaloid fraction of the extract

Seven (7) sterile petri dishes were labelled according to the concentrations of the alkaloid

fraction to be used. Mannitol salt agar solution was prepared and 20 ml of the solution was measured and transferred into the labelled sterile petri dishes. The agar was allowed to cool and solidify before inoculating the individual agar plates with fresh overnight broth culture of the different samples of the MRSA using sterile swab sticks. The culture plates were incubated for 24 hours at 37°C. Thereafter results were observed and recorded with respect to the zones of inhibition.

Determination of minimum inhibitory concentration of the alkaloid fraction of *Carica papaya* leaves extract

The determination of the minimum inhibitory concentrations of the extract was carried out to determine the lowest concentration of the *Carica papaya* that can inhibit the visible growth of Methicillin Resistant Staphylococcus Aureus. This process was carried out using agar diffusion method. Mueller Hinton agar was prepared and transferred into each of the test tubes containing the individual alkaloid fraction concentration. These were transferred into 7 sterile petri dishes labelled according to the sample of MRSA used

as well as the concentration of the extract. The agar was allowed to cool and solidify before inoculating with the different samples of methicillin resistant *Staphylococcus aureus* using a sterile wire loop. The culture plates were incubated for 24 hours at 37°C. Results obtained after 24 hours were recorded.

Calculation of percentage yield of the extract

Weight of the powdered plant material= 400g

$$\text{percentage yield} = \frac{\text{final weight}}{\text{initial weight}} \times 100$$

Weight of methanol extract = 46.3g

$$\text{percentage yield} = \frac{46.3}{400} \times 100 = 11.57\%$$

Results

From the experiment conducted, the results obtained were determined by measuring the zones of inhibition of the bacteria growth and the minimum inhibitory concentration of the alkaloid fraction of the *Carica papaya leaf extract*. The crude extract and the alkaloid fraction of the extract were tested against the Methicillin Resistant *Staphylococcus Aureus*.

Table 1: Percentage yield of the extract

Extract	Weight of extract	Percentage yield
Methanolic extract	46.3g	11.575%

Table 2: Cloxacillin susceptibility disc test result on different samples of *Staphylococcus aureus*

S/No	Samples	Mean zone of inhibition ± SD (mm)	Inference
1	A	9±1.414	Resistant
2	B	10±2.828	Resistant
3	C	10±1.414	Resistant
4	D	4±5.657	Resistant
5	E	0	Resistant
6	F	10±0.00	Resistant
7	G	4±5.657	Resistant
8	H	Nil	Susceptible

Key: Sample A - G are methicillin resistant while sample H is not.

Table 3: Antimicrobial activity of *Carica papaya* crude extract

S/No	Samples	Zones of Inhibition ± SD (mm)					
		500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	15.625mg/ml
1	A	5±0.000	4±0.000	4±1.414	3±0.000	-	-
2	B	-	-	-	-	-	-
3	C	-	-	-	-	-	-
4	D	4±0.000	4±0.000	-	-	-	-
5	E	6±1.414	4±0.000	-	-	-	-
6	F	7±1.414	5±1.414	-	-	-	-
7	G	8±1.414	4±0.000	-	-	-	-

Key: - Represents absence of any zone of inhibition
SD- Standard Deviation.

From table 3 above, samples **B** and **C** showed no zone of inhibition regardless of the concentration of the extract used. The concentration 500mg showed the highest zone of inhibition and the sample **A** is more susceptible to the extract compared to other samples since four different concentrations showed zones of inhibition.

Table 4: Antimicrobial activity of alkaloid fraction of *Carica papaya* leaves

S/N	Samples	Zones of Inhibition ± SD (mm)					
		500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	15.625mg/ml
1	A	11±0.000	7±1.414	4±0.000	4±0.000	-	-
2	B	9±0.000	5±1.414	3±0.000	-	-	-
3	C	6±1.414	4±0.000	4±0.000	-	-	-
4	D	8±1.414	6±0.000	4±0.000	-	-	-
5	E	9±0.000	6±1.414	4±0.000	-	-	-
6	F	8±0.000	6±1.414	-	-	-	-
7	G	6±1.414	4±0.000	3±0.000	-	-	-

Key: - Represents absence of inhibition

This table shows the diameter of the zones of inhibition(mm) of the alkaloid fraction of *Carica papaya* leaves. All samples of the MRSA used showed zones of inhibition to different concentrations of the fraction. 500mg/ml concentration gave the highest zone of inhibition in all samples.

Table 5: Minimum Inhibitory Concentration of Alkaloid Fraction of *Carica papaya* Leaves' Extract Against samples of MRSA

S/No	SAMPLES	Concentrations (mg/ml)						
		500	250	125	62.5	31.25	16.625	7.8125
1	A	-	-	-	+	+	+	+
2	B	-	-	-	+	+	+	+
3	C	-	+	+	+	+	+	+
4	D	-	-	+	+	+	+	+
5	E	-	+	+	+	+	+	+
6	F	-	-	+	+	+	+	+
7	G	-	+	+	+	+	+	+

Key: - Represents absence of growth of the microbe
 + Represents growth of microbes
 MIC Represents Minimum Inhibitory Concentration

The table above shows the minimum inhibitory concentrations of the alkaloid fraction of *Carica papaya* leaves' extract against different samples of MRSA. For the samples A and B, the Minimum Inhibitory Concentration is 125mg/ml. The minimum inhibitory concentration for the MRSA samples C, E, and G is 500mg/ml and that of D and F is 250mg/ml.

Discussion

The presence of bioactive substances has been reported to confer resistance to plants against bacteria, fungi and pests, and therefore explains the demonstration of antibacterial activity by the plant extract used in this study (Srinvasan et al., 2001). The leaves of *Carica papaya* has been shown to contain phytochemicals which confer its antimicrobial properties and the phytochemical analysis of the leaf extract of *Carica papaya* showed the presence of Alkaloids, Phenols, Sugar, Proteins and Tannins after subjecting the crude extracts to tests using specific reagents/solutions. Aravind et al. (2013) reported that the numerous therapeutic values of papaya, are due to the high content of the Vitamins A, B and C, proteolytic enzymes like papain and chymopapain that have antiviral, antifungal and antibacterial properties. From this research conducted, several bioactive components were absent in comparison to other research works conducted with extracting solvents other than methanol for example that conducted by Umar et al. (2018), this may be attributed to the difference in solubility of the bioactive component and this can result to different levels of action on the test bacteria.

Phytochemical analysis of *Carica papaya* leaves revealed the presence of phenols in the crude extract, this may account for the activity against the samples of methicillin resistant *Staphylococcus aureus* since phenols possess antimicrobial activity. The presence of alkaloid was also recorded, and upon fractionation and antimicrobial activity test, it was observed that the alkaloid fraction also possessed a high level of activity against the samples of the organism used. Tannin is also a known metabolite that shows activity against microbes.

As seen in table 2, the methanol extract of the *Carica papaya* leaves shows the presence of Alkaloid, Phenol, Sugar, Protein, and Tannin. Terpenoid, Saponins Flavonoid, and Quinones were not present based on the test carried.

The methicillin resistant *staphylococcus aureus* samples were determined using cloxacillin susceptibility discs and the concentration of the antibiotic used was 5µg. Samples which showed zones of inhibition were carefully observed and the zones of inhibition were measured, those which had average readings less than 10mm were tagged as resistant and were employed for

further analysis whereas those with zones of inhibition above 10mm were considered unsuitable for use. The samples A to G as shown in the table 4 were tagged as resistant since their zones of inhibition were equal to or below 10mm. From the table, the samples E, D and G showed least zones of inhibition indicating their low levels of resistance to the antibiotic.

The antimicrobial property of the leaves of *Carica papaya* was measured in terms of the zone of inhibition observed. Pilot tests were carried out to determine whether the crude extract possessed antimicrobial properties. From the result obtained, the growth of the different samples of Methicillin Resistant *Staphylococcus aureus* were inhibited to an extent showing that the extract possesses antimicrobial properties. From the result obtained, certain biochemicals which show activity against methicillin resistant *staphylococcus aureus* are present in the extract. Table 3 shows the activity of the crude extract of *Carica papaya* leaf, the highest concentration (500mg/ml) showed the greatest activity in five out of seven samples followed by the concentrations 250mg/ml, 125mg/ml and the least 62.5mg/ml. The concentrations 31.25mg/ml and 15.625mg/ml showed no observable activity against the test organism (MRSA) due to their increased level of dilution. The samples B and C were not inhibited by any of the crude extract concentrations used. This is a strong pointer to the high level of resistance of the samples. The zones of inhibition varied with respect to the samples used as well as the concentration of the crude extract used.

The alkaloid fraction of leaf sample was tested against the samples of the test microorganism (MRSA) and the results observed were recorded in table 4. As seen from the table, a greater level of activity was recorded compared to that observed after using the crude extract. The concentrations; 31.25mg/ml, 16.625mg/ml and 7.8125mg/ml showed no observable activity against the samples used. The concentration 62.5mg/ml only showed activity against the sample A. The samples B and C which were initially resistant to the crude extract were observed to be susceptible to various concentrations of the alkaloid extract, this shows

the essence of fractionation (this was done to show the fraction with increased activity against the bacterium). The concentration 62.5mg/ml showed no activity against all samples except sample A. The largest zone of inhibition was observed while using sample A and the concentration used was 500mg/ml. The smallest zone of inhibition was observed when using the sample, A and the concentration used was 62.5mg/ml.

The factors influencing the result obtained include; Concentration of the bacteria spread on the agar, Incubation temperature, Incubation time, pH, agar depth size of the inoculated organism, reading of the zone and so on.

The table 5 shows the minimum inhibitory concentration of the alkaloid fraction which is defined as the least concentration of the extract (antimicrobial) agent that is required to stop the growth of a given sample of microorganism. From the table, the least concentration that could inhibit the growth of the samples A and B of methicillin resistant *staphylococcus aureus* was 125mg/ml whereas the sample D and F were inhibited by 250mg/ml as the minimum inhibitory concentration. The minimum inhibitory concentration for the samples C, E and G was 500mg/ml.

Using the sample, A, in comparing the activity of cloxacillin to that of the crude extract of *Carica papaya* and the alkaloid fraction, the average zone of inhibition observed while using cloxacillin 5 μ g/ml concentration was 9mm, whereas the highest zone of inhibition seen while using the crude extract concentration 500mg/ml was 5mm followed by 4mm, 4mm and 3mm for the concentrations 250mg/ml, 125mg/ml and 62.5mg/ml respectively. Cloxacillin is more potent in action in comparison to the crude extract despite its low concentration. In comparison to the alkaloid fraction, cloxacillin's activity in terms of the zone of inhibition is surpassed by that of the alkaloid fraction although this effect is low since a much higher concentration of the alkaloid fraction was required to achieve that level of activity-500mg/ml gave a zone of inhibition of 11mm followed by 7mm, 4mm, and 4mm by the

concentrations 250mg/ml, 125mg/ml and 62.5mg/ml respectively, these were higher than those observed in the crude extract indicating the importance of fractionation of the components of the leaves of *Carica papaya*

The samples B and C were totally resistant to the different concentrations of the crude extract of *Carica papaya*, since no zone of inhibition was observed. While using cloxacillin, the zone of inhibition observed was 10mm in both sample B and C. The alkaloid fraction concentrations of 500mg/ml, 250mg/ml and 125mg/ml caused zones of inhibition of 9mm, 5mm and 3mm respectively for the sample B. For that of sample C, the alkaloid fraction concentration of 500mg/ml, 250mg/ml and 125mg/ml showed the zones of inhibition of 6mm, 4mm, and 4mm respectively which indicates a higher level of activity in comparison to the crude extract but a lower activity level in terms of concentration and measure of the zones of inhibition in comparison to cloxacillin.

For the sample D, while using cloxacillin, the zone of inhibition observed was 4mm and that observed while using the plant crude extract at concentrations, 500mg/ml and 250mg/ml were 4mm each, showing comparable activity. This is poor since a much higher concentration of the extract is required to achieve the same effect measured after the use of cloxacillin. In comparison to the alkaloid fraction with zones of inhibition of 8mm, 6mm, and 4mm for the concentrations 500mg/ml, 250mg/ml and 125mg/ml respectively, the alkaloid fraction shows a higher level of activity to that of cloxacillin and the crude extract.

The sample E showed no zone of inhibition after introducing cloxacillin whereas zones of inhibition, 6mm and 4mm were observed after using the plant extract concentration 500mg/ml and 250mg/ml respectively. The concentration of alkaloid fraction of the *Carica papaya* leaves that showed activities against the samples are 500mg/ml, 250mg/ml, 125mg/ml and the corresponding zones of inhibition were 9mm, 6mm and 5mm respectively, this shows a higher level of activity in comparison to cloxacillin. Factors responsible for the result include; nature

of the sample used, the rate of diffusion of the antibiotics as well as the thickness of the agar used.

For the sample F, the zone of inhibition measured after using cloxacillin was 10mm this is greater than that observed after using the alkaloid fraction of the *Carica papaya* leaf extract of concentration; 500mg/ml and 250mg/ml which gave the zones of inhibition of 8mm and 6mm respectively. Other concentrations showed no observable activity. For the crude extract, the zones of inhibition recorded were 7mm and 5mm for the concentrations 500mg/ml and 250mg/ml respectively.

The concentrations 500mg/ml and 250mg/ml of the crude extract produced zones of inhibition 8mm and 4mm respectively after testing its activity against the sample G. Cloxacillin produced a mean zone of inhibition of 4mm at a concentration of 5 μ g whereas the alkaloid fraction with concentration levels 500mg/ml, 250mg/ml and 125mg/ml, the zones of inhibition measured were; 6mm, 4mm and 3mm respectively although the highest alkaloid fraction but this is still low in comparison to that of the cloxacillin due to the low concentration level of the cloxacillin.

The table 5 shows the minimum inhibitory concentrations of the alkaloid fraction of *Carica papaya* leaves' extract against different samples of MRSA. For the samples A and B, the Minimum Inhibitory Concentration is 125mg/ml. The minimum inhibitory concentration for the MRSA samples C, E, and G is 500mg/ml and that of D and F is 250mg/ml.

Conclusion

From this study, the alkaloid fraction demonstrated a higher level of activity in comparison to the crude extract of the *Carica papaya* leaves. The crude extract exhibited a mild degree of inhibition on the various samples of MRSA except for samples B and C. The alkaloid fraction of the *Carica papaya* leaves show a higher level of activity against the MRSA samples used with the highest activity seen in sample A with 11mm zone of inhibition at a concentration of 500mg/ml.



A further bioassay guided fractionation and other analytical procedures as well as purification processes, need to be carried out to obtain the most active component which can inhibit the organism at a lower concentration.

As a result of the emergence of resistance to antibiotics which presents a serious threat to successful treatment of infectious agents such as MRSA, *Carica papaya* leaf extract (alkaloid fraction) could be used as an adjunct therapy with antibiotics such as penicillin, aminoglycosides, cephalosporin, and so on to combat infections caused by MRSA.

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