Antibacterial Activity of Fractionated Extracts of Carica papaya Leaves and Stem Bark against Clinical Isolates of Methicillin Resistant Staphylococcus aureus (MRSA)

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Abstract
The research was aimed to determine the phytochemical screening and antibacterial activity of fractionated leaves and stem bark extracts of Carica papaya on six different clinical isolates of Methicillin Resistant Staphylococcus aureus (MRSA) recovered from infected patients attending Murtala Muhammad Specialist Hospital. Preliminary phytochemical screening of the extracts showed the presence of alkaloids, saponin, phenol, flavonoids, protein and amino acid, reducing sugar, anthraquinone steroid and terpenoid. Using agar well diffusion method for determination of antibacterial activity of the extracts, the results showed that the fractionated leaves extracts showed higher activity against the isolates (with average zone of inhibition of 16.40±1.55 mm) with n-hexane fraction showing higher antibacterial activity (18.23±1.12 mm) than ethyl-acetate (15.35±1.04 mm) and n-butanol (13.33±0.95 mm) fractions while the isolates were resistant to stem bark extract. Statistical analysis of the results showed that ISOL. 3 is the most susceptible to the extracts used covering an average zone of inhibition of 19.83 ±1.10 mm followed by ISOL 6 (19.66±1.06 mm). Least zone of inhibition was recorded in ISOL 5 (09.80±0.31 mm) which is resistant to the extracts used. There is statistical significant different on the activity of the extracts and susceptibility of the organisms against the extracts at p<0.05. Based on the findings of this research, the ethno botanical application of the plant (Carica papaya) is justified.

Keywords: Antibacterial activity; Carica papaya; Phytochemical; Methicillin resistant Staphylococcus aureus

Abbreviations: DMSO: Dimethylsulphoxide; MRSA: Methicillin Resistant Staphylococcus aureus; TLC: Thin Layer Chromatography

Introduction
Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity [1]. More recently, medicinal plant extracts were developed and proposed for use in food as natural antimicrobials [2-4]. In recent times, research interest for active chemical agents against MRSA, especially from indigenous medicinal plants resources has received the attention of pharmacists across the globe [5-7]. However, little or no work has been done on the effects of papaya extracts on methicillin resistant Staphylococcus aureus in Northern Nigeria.

The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors 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 appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [8]. There is no plant that does not have medicinal value. The active components are normally extracted from all plant structures, but the concentrations of these components vary from structure to structure. However, parts known to contain the highest concentration of the principles are preferred to therapeutic purposes and it can either be the leaves, stems, barks, roots, bulbs, corms, rhizomes, woods, flowers, fruits or the seeds [9].

Carica papaya L. belongs to the family of Caricaceae, and several species of Caricaceae have been used as a remedy against a variety of diseases [10]. Carica papaya L. is commonly called pawpaw
(English), Gwanda (Hausa), Illebe (Yoruba) or Okoegbe (Igbo) [11]. It is a mono-sexual plant of Central American origin [11]. Besides the fruit been edible, it has been reported that the roots and the leaves have been used as antihelminthes and for the treatment of infections of bacterial origin [12]. Papaya leaf extracts have phenolic compound and caffeic acid [13]. Carica Papaya plant produce natural compound in leaf and bark as well as twig tissues that poses both highly anti-tumor and pesticide properties [14]. The aim of the study is to determine the antibacterial activity and phytochemical constituents of fractionated leaves and stem bark extract of Carica papaya against clinical isolates of Methicillin Resistant Staphylococcus aureus MRSA.

Materials and Methods

Ethical approval

Ethical approval (issue number HMB/GEN/488/VOL. 1) was obtained from the Murtala Mohammed Specialists Hospital (MMSH), Kano based on the consent of the Hospitals Ethical Committees.

Experimental microorganisms

The experimental organisms (six different isolates of Methicillin Resistant Staphylococcus aureus) were isolated from clinical samples of high virginal swab, wounds and skin of patients from hospital patients presenting symptoms of Staphylococcus aureus -associated diseases attending Muratla Mohammed Specialists Hospital Kano, Nigeria. The isolates were identified by standard method [15]. The pure culture of the confirmed isolates were preserved on Nutrient agar slants, labeled, transported to Microbiology Laboratory of Kano State University of Science and Technology Wudil and stored in refrigerator at 4 ºC for subsequent tests.

Collection of plant materials

Fresh green leaves and stem bark of Carica papaya were collected from Biological garden of Ahmadu Bello University School of Basic and Remedial Studies Funtua. The plant materials were carried to Herbarium in the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria where they were authenticated. A voucher number was issued as 307. The plant materials were washed thoroughly 5 times in sterile distilled water. Then air-dried under shade at room temperature for 14 days and pulverized to finely powdered form using pestle and mortar as described by Ali et al. [16].

Preparation of extracts

The crude extract from leaves and stem bark of Carica papaya was prepared according to the method proposed by Alabi et al. [17]. Fifty grams of powdered sample of plant’s parts were extracted exhaustively (cold maceration) with distilled water and ethanol for 7 seven days. The extracts were filtered using Whatman No. 2 filter paper, and concentrated at reduced pressure in water bath (for aqueous extract) and rotary evaporator (for ethanol extract) at 40 ºC to afford the various crude extracts of the plant’ s parts. The samples were kept in the refrigerator at 4 ºC until use.

Fractionation of the crude extract

The partitioning procedure was done using solvents of three different polarities. 80g of the Crude extract was dissolved in 600ml distilled water in a measuring cylinder, shaken and allowed to stand for 10 minutes. The mixture was transferred in to separation funnel followed by addition of normal hexane, the mixture were allowed to stand for the some 15 minutes for partitioning to take place. The tap from the separation funnel was released and the fraction collected inside a sterile bottle. The same procedure was repeated for normal n-butanol and ethyl acetate. Each fraction was transferred into evaporation dish and evaporated using water bath at 45 ºC. After evaporation each fraction was weighed, recorded and transferred into sterile bottle for sensitivity test. A concentration of 400mg/ml was prepared for each of the fraction by weighing 4g of the plant extract in 10ml of 10% DMSO as stock solution.

Thin Layer Chromatography (TLC) of the fractions

Thin layer Chromatography was done using TLC prepared plate as described by Sherma & Fried [18]. Small amount of the fractions was dissolved in distilled water in a separate test tube. Each fraction that of the extract, Hexane, Ethyl-acetate, butanol and aqueous fraction were spotted on TLC prepared plate using capillary tube. The TLC plates was then placed inside TLC tank containing two different solvent system, hexane ethyl acetate 3:7 and butanol Acetic acid: water 3:1:1. After 30 minutes, the TLC plates were removed and allowed to dryness, it then sprayed with detecting agent of 0.5ml of para-anisaldehyde in 50ml of glacial acetic acid and 1ml of 97%. Concentrated Tetraoxosulphate (VI) acid (H2SO4) and heated inside ovum at 105 ºC. The movement of the spots was viewed and their respective Retention Factor (Rf) values were recorded.

Phytochemical Screening

The extracts were subjected to various phytochemical tests to identify the phytochemical constituents present using standard methods as described by Sofowora [19]. Trease & Evans [20]. Phytochemical screening was performed to test for alkaloids, saponin, phenol, flavonoids, Protein and amino acid, reducing sugar, tannin, anthraquinone steroid and terpenoid.

Determination of antibacterial activity of the extracts

Antibacterial activity of the aqueous and ethanolic n- hexane, n- butanol and ethyl acetate fractionated extracts of the leaf and stem bark of Carica papaya were determined using agar well diffusion method as described by Aida et al. [21]. For the test, Muller-Hinton agar plates were swabbed with standard test isolates (0.5 McFarland), two wells were made on the surface of the agar using 6mm sterile steel borer and the wells (6mm) were filled with 400mg/ml concentration of 0.1ml of each extract and 200mg/ml of Tetracycline as a positive control. The cultures were incubated at 37 ºC for 24 hours. The antibacterial potential of test extracts was determined on the basis of diameter of zone of inhibition around the wells as described by Sumitra & Sharma [22].
Statistical Analysis

The zone of inhibition produced by the isolates against the extracts used was analyzed using One-Way ANOVAs using statistical program SPSS 21.0 (Statistical Package for the Social Sciences). Significance level for the differences was set at p<0.05.

Results

Sources of the isolates

Six (6) different isolates of Methicillin Resistant *Staphylococcus aureus* were isolated from clinical samples of high virginal swab (n=1), wounds (n=3) and skin (n=2) of patients from hospital patients presenting symptoms of *Staphylococcus aureus*-associated diseases attending Murtala Mohammed Specialists Hospital Kano, Nigeria (Table 1).

Table 1: Various sources of the isolates used.

| S/N | Source            | Code  |
|-----|-------------------|-------|
| 1   | High vaginal swab | ISOL 1|
| 2   | Skin              | ISOL 2|
| 3   | Skin              | ISOL 3|
| 4   | Wound             | ISOL 4|
| 5   | Wound             | ISOL 5|
| 6   | Wound             | ISOL 6|

Phytochemical screening

Table 2: Phytochemical constituents of leaves and stem of *Carica papaya*.

| S/N | Phytochemicals | Leaf Extract | Stem Bark Extract |
|-----|----------------|--------------|-------------------|
| 1   | Alkaloid       | +            | +                 |
| 2   | Saponin        | +            | +                 |
| 3   | Phenol         | +            | -                 |
| 4   | Flavonoids     | +            | +                 |
| 5   | Protein and amino acid | +        | -                 |
| 6   | Tannin         | -            | -                 |
| 7   | Reducing sugar | +            | -                 |
| 8   | Anthraquinone  | +            | -                 |
| 9   | Steroid        | +            | -                 |
| 10  | Terpenoid      | +            | -                 |

The phytochemical screening of the active phytochemical constituents of the extracts is presented in Table 2. Leaf extract contain alkaloids, saponin, phenol, flavonoids, Protein and amino acid, reducing sugar, anthraquinone steroid and terpenoid except Tannin, while stem extract contain only three phytochemicals Alkaloid, Saponin and Flavonoids. The result showed that *Carica papaya* leaves extract contain more phytochemicals than the stem bark extract.

Antibacterial activity of fractionated leaves extracts

The antibacterial activity of the fractionated plant leaf extract (at 400mg/ml) against the 6 isolates of MRSA is presented in Table 3. The result shows that highest zone of inhibition was shown by n-hexane, ethyl acetate and butanol extract.

Table 3: Antibacterial activity of the fractionated leaf extracts.

| S/N | Fract Plant Extract | ISOL 1 | ISOL 2 | ISOL 3 | ISOL 4 | ISOL 5 | ISOL 6 |
|-----|---------------------|--------|--------|--------|--------|--------|--------|
| 1   | Ethanol(n-hexane)   | 20.00a | 17.00a | 21.00a | 19.00a | 13.00a | 22.00a |
| 2   | Ethanol(n-butanol)  | 14.00a | 17.00a | 20.00a | 16.00a | 08.00a | 21.00a |
| 3   | Ethanol(ethyl acet.)| 19.00a | 18.00a | 22.00a | 18.00a | 10.00a | 21.00a |
| 4   | Aqueous(n-hexane)   | 19.00a | 16.00a | 22.00a | 15.00a | 16.00a | 19.00a |
| 5   | Aqueous(n-butanol)  | 13.00a | 13.00a | 15.00a | 13.00a | 06.00a | 17.00a |
| 6   | Aqueous(ethyl acet.)| 19.00a | 14.00a | 19.00a | 15.00a | 06.00a | 18.00a |

Key: 06.00 = No zone of inhibition, Values having different superscript on the same row are considered significantly different at p<0.05

Antibacterial activity of fractionated stem bark extracts

The antibacterial activity of the fractionated stem bark extract (at 400mg/ml) against the 6 isolates of MRSA is presented in Table 4. The result shows that the highest zone of inhibition was shown by n-hexane extract with zone of inhibition of 22mm. lowest zone of inhibition was recorded in n-butanol (8mm). Isolate 3 and 6 were found to be more sensitive to the extract while isolate 5 was more resistant.

Table 4: Antibacterial activity of the fractionated stem extracts.

| S/N | Fract Plant Extract | ISOL 1 | ISOL 2 | ISOL 3 | ISOL 4 | ISOL 5 | ISOL 6 |
|-----|---------------------|--------|--------|--------|--------|--------|--------|
| 1   | Ethanol(n-hexane)   | 06.00a | 06.00a | 09.00a | 10.00a | 06.00a | 06.00a |
| 2   | Ethanol(n-butanol)  | 09.00a | 06.00a | 09.00a | 06.00a | 06.00a | 06.00a |
| 3   | Ethanol(ethyl acet.)| 11.00a | 06.00a | 10.00a | 09.00a | 06.00a | 06.00a |
| 4   | Aqueous(n-hexane)   | 06.00a | 06.00a | 09.00a | 09.00a | 06.00a | 06.00a |
| 5   | Aqueous(n-butanol)  | 09.00a | 06.00a | 06.00a | 08.00a | 06.00a | 06.00a |
| 6   | Aqueous(ethyl acet.)| 10.00a | 06.00a | 10.00a | 06.00a | 06.00a | 06.00a |

Key: 06.00 = No zone of inhibition, Values having different superscript on the same row are considered significantly different at p<0.05

Discussion

The result for phytochemical screening of *Carica papaya* showed that the plants contained some phytochemical compounds which possess good antimicrobial properties on the test clinical isolates used in the study. The phytochemical analysis of the plant showed that the leaves contain Anthraquinones, phenols, glycoside, carbohydrates, and flavonoids, which are known to have strong antioxidant activity. The leaves and stems of *Carica papaya* have been reported to be useful in the treatment of various ailments such as malaria, typhoid, dengue, and parasitic infections, among others.
amino-acid, terpenoid, reducing sugar, Saponin, Tannin, Alkaloids and Flavonoids. On the other hand, the stem extract contains Alkaloid, saponin, flavonoids and reducing sugar. This finding can be attested to the work of Sikanda et al. [23] who also reported similar finding and also stated the effect of these phytochemicals as a good antimicrobial agent on different test organism. Doughari et al. [11] reported the anti-bacterial effect of the extract of C. papaya on various bacterial isolates including Staphylococcus aureus, Salmonella typhi and Bacillus cereus. Although the mechanism of action of this extract is not understood, it has been proposed that its action against the bacteria and fungi may be due to the inhibition of cell wall formation in the cell resulting in a leakage of cytoplasmic constituents by the bioactive components of the extract [24,25].

In addition, bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explain the demonstration of antibacterial activity by the plant extracts used in this study [26]. In these regard, Aravind et al. [27] reported that the many benefits of papaya, are due to the high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain that have antiviral, antifungal and antibacterial properties while phytochemical compounds such as tannin coagulate the wall proteins, saponins facilitated the entry of toxic material or leakage of vital constituents from the cell [28].

Flavonoids inhibit the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins, more lipophilic flavonoids disrupt cell wall integrity [29] or microbial membranes [30] at low concentrations. The existence of Saponin supports the fact that papaw has cytotoxic effect such as permealization of the intestine as Saponins are cytotoxic [31]. Alkaloids are the most efficient therapeutically influential plant substance. Pure natural and synthetic derivatives of alkaloids are used as a basic medical agent because of their analgesic, antispasmodic and antibacterial properties [32].

The presence of Alkaloid in the pawpaw shows that this plant can be an effective anti-malaria agent since alkaloid consists of quinine, which is anti-malaria [33]. Marchese & Shito [34], Poole [35] reported the sensitivity of the microbial strains to both the plant extracts and to synthetic antibiotics, and observed that the plant extracts compete favorably with the drugs and can be used as an alternative to the antibiotics as the zones of inhibition shown were very comparable and the extracts have lesser side effects which are often associated with the use of antibiotics. Also, the issue of resistance to these extracts cannot arise as is found with antibiotics [36].

The present study showed that the leaves of Bacillus cereus papaya possess antimicrobial potential against MRSA. In line with the present finding, several other studies [37-39] have reported Cereus papaya leaves have antimicrobial potentials and have significant antibacterial activity in various extracts from different tree's parts. The antimicrobial activity of fractionated extracts of Carica papaya leaves showed zone of inhibition against the isolates tested. The extract of n-hexane fraction exhibited highest zone of inhibition with average of 18.23mm at 400mg/ml while n-butanol and ethyl-acetate fraction has an average of 13.33 and 15.60mm at 400mg/ml respectively. The result of his study supported that of Yahaya et al. [40] who the aqueous and ethanol extract of Carica papaya leaves active against Escherichia coli, S. typhi, Pseudomonas aeruginosa and Staphylococcus aureus.

Conclusion

According to this study, ethanol extracts demonstrated a higher activity than the aqueous extracts in both extracts. The better efficacy of the ethanol extract as against the aqueous extract may be because different solvents have different polarities, hence different degrees of solubility for the various phytoconstituents. It was also found that leaves extracts of the plant possessed higher antibacterial efficacy when compared to stem bark extracts. Phytochemical screening of the extracts showed the presence of anthraquinone, phenols, glycoside, and amino-acid, terpenoid, reducing sugar, Saponin, alkaloids and flavonoids. The phytochemicals are responsible for the antibacterial activity of the extracts.

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