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Antibacterial Activity of *Carica papaya* against Methicillin resistant *Staphylococcus epidermidis* Isolated from Wards Surfaces of Kampala International University TeachingHospital, Bushenyi, Uganda

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Abstract

This study aimed at determining the antibacterial activity of *Carica papaya* against Methicillin resistant *Staphylococcus epidermidis* isolated from doorknobs, bed rails, floors and walls of Surgical, Medical, Maternity, Pediatrics, Accident and Emergency, Semiprivate and Private wards of Kampala International University Teaching Hospital. The bacteria were isolated from the wards surfaces and identified using biochemical tests, Desferroxiomine and Fosfomycin antibiotics. Disc diffusion method was used to detect methicillin resistance in *S. epidermidis* using Cefoxitin (30 µg) disc. Fresh leaves and seeds of *C. papaya* was processed and extracted using standard methods. Antibacterial activities of the methanol, acetone and aqueous crude extracts were assayed using the agar well diffusion method. Phytochemical analysis, Minimum Inhibitory and Bactericidal concentration of the crude extracts were determined using broth dilution methods. Both *C. papaya* leaf and seed crude extract exhibited antibacterial activity against MRSE with MICs and MBCs ranges of 250 to 31.3mg/ml and 125 to 31.3 mg/mL for leaf and seed extracts respectively. This study concludes that *C. papaya* leaf and seed crude extracts were effective against Methicillin resistant *S. epidermidis.*

Introduction

Staphylococcus epidermidis (*S. epidermidis*) is one of the human normal flora, typically the skin flora, and less commonly the mucosal flora (1). *S. epidermidis* possesses a wide variety of surface expressed molecules, some of which are likely to have important roles in the survival and adhesion of the skin (2). Transmission of *S. epidermidis* in the health care setting arises through contact with contaminated surfaces in the environment (3). *S. epidermidis* have the capability to survive within hospital settings surroundings on medical devices and medical equipment such as patient care equipment, uniforms, computer keyboards, cellular phones, bed rails, door knobs, table tops and identification badges for weeks to months (4). *S. epidermidis* and similar organisms have the ability to adhere to the surfaces, as a result of their unique pathogen-host-environment relationships (5).

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Staphylococcus epidermis is one of the major causes of nosocomial infections, which affects two million people annually, with a 25 % mortality rate, and prolong hospital stay among 5 to15% of patients worldwide (6,7). *S. epidermidis* strains circulating in hospitals have been established to be methicillin-resistant (8). The methicillin-resistance to *S. epidermis* are being usually due to the mica gene, which is carried by staphylococcal cassette chromosome mec (SCCmec), and produces a Penicillin binding protein 2A (PBP2A) with low affinity for ß-lactams (9). A previous study recommends the use of Vancomycin, Gentamicin, Cefazolin, Linezolid and Telavancin for the treatment of Methicillin-resistant *S. epidermidis* (10). However, most of the antibiotics recommended are the second line drugs and are also expensive; hence a need for an alternative measure against infections, especially from natural sources such as Ethno-medicinal plants.

Medicinal plants such as *Carica papaya (C. papaya*) are rich sources of medicine and can provide possible inexpensive alternatives in the treatment of resistant microbial strains, due to the presence of a multitude of phytochemical compounds which are linked to antimicrobial activities (11,12). *C. papaya* L. (Pawpaw), which belongs to the family Caricaceae, is a medicinal plant recognized as an effective natural medicine in controlling both oedema and inflammation associated with surgical operations (13). Phytochemicals, such as tannins, alkaloids and phenolic compounds present in different parts of *C. papaya*, have been shown to treat different ailments (14). The leaf and seed extracts of *C. papaya* has been reported to inhibit growth of several pathogens, including Coagulase-positive *S. aureus* and Coagulase-negative Staphylococci and also used as soap substitute for the treatment of skin infections (15,16). The effect of *C. papaya* seeds extracts on *S. epidermidis,* however, has not been widely explored. Hence a need to evaluate the antibacterial activity of *C. papaya* on *S. epidermidis* isolated from Hospital surfaces.

Materials and methods

Study area and Sampling site : This study was conducted at the Faculty of Biomedical Sciences and School of Pharmacy, Kampala International University. Isolation and Identification of Methicillin resistant *S. epidermidis* and determination of antibacterial activity of *C. papaya* was conducted at the laboratory of Microbiology department, while extraction and phytochemical screening of *C. papaya* leaves and seeds were performed at the laboratory of Pharmacology department. The plant samples were collected from Kigondo village in Ishaka municipality, Ishaka-Bushenyi, Uganda (GPS location latitude 050'0"S, Longitude 31Ú33'0"E). The Methicillin-resistant *S. epidermidis* were isolated from wards surfaces (bed rails, doorknobs, floors and walls) of Kampala International University-Teaching Hospital by swabbing the surfaces using a sterile cotton swab and brought to the department of Microbiology and Immunology laboratory within the University for Identification.

Isolation and identification of Methicillin-resistant Staphylococcus epidermidis : Swabbed samples of the bacteria were collected from wards surfaces (door knobs, bedrails, floor and walls) of Kampala International University Teaching Hospital (KIU-TH) and transported to the Microbiology laboratory at the Department of Microbiology and Immunology using transport media in an ice box cooler. The samples were inoculated on a sterile agar plates containing Mannitol salt agar. Pinkish colonies were sub-cultured on new Mannitol salt agar plates and confirmed using microscopy, catalase and coagulase test (17). The suspected colonies were further confirmed using Desferroxiomine (200 µg) and Fosfomycin (200 µg) antibiotics and affirmed with Cefoxitin (30 ìg) antibiotics (18).

Collection and Identification of plant samples : The samples of *C. papaya* which is commonly known as papari in Luganda were taken to a Botanist at the Department of Biology and science laboratory technology at Mbarara University of Sciences and Technology (MUST), Uganda, for identification. Thereafter, fresh leaves and seeds (from ripe pawpaw) were collected from Kigondo village early in the morning and transported in a sterile nylon bag to the Pharmacology laboratory, Department of Pharmacology, Kampala International University Western Campus (KIU-WC) where they were shade-dried and extracted (19).

Extraction of the plant samples : Extraction was carried out using the maceration method as described by20 Gideon (20). One hundred grams (100 g) of the leaves and seeds powder was weighed separately. Each was put in three different beakers (1000 mL size) and dissolved in 500 mL of absolute methanol, acetone and distilled water, with a polarity index of 5.1, 4.1 and 10.2, respectively. The mixture was allowed to mix for 48 hours, with frequent shaking using a vibratory sieve shaker to avoid pouring or evaporation of the mixture before extraction is complete. The crude extracts were filtered using a clean cotton cloth, followed by use of Whatman filter paper number 1. The filtrate was distilled and then evaporated to remove the solvent.

Determination of percentage yield extract : The percentage yield extract was obtained using the formula: W_2-W_1/W_0× 100

Where: W_2 is the weight of the extract and the container, W_1 the weight of the container alone, and W_0 the weight of the initial dried sample (21).

Phytochemical screening of the crude extracts : The crude extracts of *C. papaya* was screened to check the presence of phytochemicals, such as: flavonoid, tannins, terpenoids, cardiac glycosides, saponins, and steroids using the standard procedures described by Gideon (20).

Antibacterial activity assay

Preparation of extracts concentration : Five hundred milligrams (500 mg) of each extract were dissolved in 1 mL of 20% Dimethyl Sulfoxide (DMSO) to obtain the concentration of 500 mg/ mL which was used for the determination of antibacterial activity as described by Gideon (20).

Antibacterial screening of the crude extracts : The antimicrobial activity of the extracts was demonstrated using the agar well diffusion method as described by Ogutu (22). Sterile Mueller Hinton agar plates were inoculated with the standardized suspension (0.5 McFarland standards) of the Methicillin resistant *S. epidermidis*. Five wells (5 mm each) were punched into the agar plates using a sterilized cork borer (5 mm). Using a micropipette, 100 ìL of both extracts were added to the first, second and third well accordingly. A concentration of 7 ìg/mL of vancomycin was prepared according to Johnson (23) and100 ìL of the prepared vancomycin was added to the fourth well as positive control while DMSO was added to the fifth wells as negative controls. Plates were incubated at 37^{Ú}C for 24 hrs. The diameter of the zone of inhibition was measured and the results interpreted according to the Clinical and Laboratory Standard Institute guidelines (24).

Determination of minimum inhibitory and bactericidal concentrations of the active crude extracts : The minimum inhibitory concentration (MIC) of the crude extracts was determined according to Ogutu (22). Two-fold serial dilution of the extract was carried out in a series of sterile tubes containing 1ml of nutrient broth to obtain different concentrations (500, 250,125, 625, 31.25 and 15.63 mg/mL). One milliliter (1 mL) suspension of the test organism compared with 0.5 McFarland standard was added to each tube. This method was modified by preparing two sterile tubes: one containing only nutrient broth and test organism without the extract, to serve as negative control; and the other containing only the broth and extract without the test organism, to serve as a positive control. Each of the tests were done in triplicate in order to minimize errors.

The diluted tubes and the plates were incubated overnight at 37ÚC. After incubation, the turbidity from each diluted tube was compared with the control tubes and the highest dilution without turbidity was considered as the MIC and interpreted in mg/mL.

The result of MIC was used to determine Minimum Bactericidal Concentration by sampling clear tubes. A loopful of broth from each clear tube was inoculated onto the nutrient agar in triplicate. The nutrient agar plate was streaked with the test organism to serve as controls. All the plates were incubated at 37ÚC for 24 hrs. After incubation the concentration at which no visible growth is seen was taken as the MBC (25).

Data analysis : The raw data was entered in excel, edited, cleaned and analysed using statistical package for social sciences (SPSS) version 21 software. One way Analysis of Variance (ANOVA) was used to compare the activity provided by leaves extract (Methanol, Acetone and Aqueous) and seeds (Methanol, Acetone and Aqueous) while t-test was used to compare the activity of Methanol leaf with that of Methanol seed extracts and Acetone leaf with that of Acetone seed extracts respectively. And p value of d"0. 05 used to determined significance between the activities of the extracts.

Ethical approval : Permission was sought from the management of Kampala International University Teaching Hospital for collecting samples from the wards surfaces. The resistant bacteria (MRSE) were handled with care following the guidelines provided by Clinical Laboratory Standard Institute (24).

Results

In the leaf and seed of *C. papaya,* methanolic crude extract gave the highest yield of 9% and 6.4%, respectively, while the aqueous gave the least yield of 5% and 4.2% respectively as shown in **Table 1.**

The phytochemical analysis of the plant extract carried out in this study revealed the presence of tannins, terpenoids, cardiac glycosides, alkaloids, phenols and triterpenoids in both leaf and seed extract. However, seed additionally had flavonoids, saponins and steroids as shown in **Table 2**.

Table 6 shows the antibacterial activity of methanol, acetone and aqueous crude extracts of *C. papaya* leaf and seed against antibiotic resistant *S. epidermidis* isolated from wards surfaces of KIU-TH. The seed crude extracts showed more activity than the leaf. Both seed and leaf aqueous crude extract had no activity while methanolic crude extract had the highest activity with an inhibition zone diameter of 23 mm and 16.5 mm respectively. Vancomycin (positive control) had an inhibition zone diameter ranging from 23 to 13 mm. The difference between the activity of the extracts from both leaf and seed is statistically significant at p-value < 0.05. However, there was no statistical difference between the activity of acetone and methanol leaf extracts at p-value = 0.6650. Furthermore, there was no significant difference between the activity of acetone seed extract and acetone leaf extract at p-value =0.0559 but the difference between the activity of methanol seed extracts and methanol leaf is statistically significant at p-value= 0.0040.

Table 4 showed the minimum inhibitory concentration and minimum bactericidal concentration of methanol and acetone crude extracts of *C. papaya* leaf. The minimum inhibitory as well as the minimum bactericidal concentrations ranges from 250 to 31.3 mg/mL respectively. The Methanolic leaf crude extract had the lowest MIC values (31.3 mg/mL) against PD23B, MT5W, SG11B and highest MIC values (125 mg/mL) against MD11B, AE48F, SG17B and SG3D. Acetone extract gave highest MIC value (125 mg/mL) against AE48F and lowest MIC values (31.3 mg/mL) against MD11B, PD19B, MD53F and AE28B. The highest MBC value (250 mg/mL) of the methanolic leaf extract was shown against SG17B while the lowest (62.5 mg/mL) was against MT13W, PD23B, PD19B, MT5W, MD53F and SG11B. Acetone extract gave highest MBC values (125 mg/mL) against MT13W, AE48F, SG17B and SG11B, and the lowest values (31.3 mg/mL) against MD11B, PD19B and MD53F.

The results on the minimum inhibitory concentration and minimum bactericidal concentration of methanol and acetone crude extracts of *C. papaya* seed was shown in **Table 5.** The minimum inhibitory and the minimum bactericidal concentrations ranged from 125 to 31.3 mg/mL respectively. The Methanolic seed

Table 1: Percentage yield of leaf and seed crude extract of *Carica papaya* in different solvents

Crude extract	**Leaf (%)**	**Seed (%)**
Methanol	9	6.4
Acetone	6.4	5.2
Aqueous	5	4.2

Table 2: Phytochemical analysis of *Carica papaya* leaf and seed crude extracts

Phytochemicals	Test performed	Leaf crude extracts			Seed crude extracts		
		ME	AE	AqE	ME	AE	AqE
Flavonoids	Lead acetate test	-	-	-	+	-	-
Tannins	Ferric chloride test	+	+	+	+	+	-
Terpenoids	Sulphuric acid test	+	+	-	+	+	-
Cardiac Glycosides	Borntragor's test	+	-	-	+	-	-
Saponins	Water test	-	-	-	-	-	+
Steroids	Chloroform test	-	-	-	+	+	-
Alkaloids	Wagner's test	+	+	+	+	+	-
Phenols	Ferric Chloride test	+	+	+	+	+	+
Triterpenes	Salkovaski's test	+	+	-	+	+	-

Key: ME= Methanol extract, **AE=** Acetone extract, **AqE=** Aqueous extract, **+** represents positive, **-** represents negative

Table 3: Antibacterial activity of leaf and seeds crude extracts of *Carica papaya* on Methicillin resistant *Staphylococcus epidermidis*

Methicillin resistant isolates of *S. epidermidis*	Ward Surfaces	Mean inhibition zone of leaf crude extracts diameter (mm)			Mean inhibition zone of seed crude extracts diameter (mm)			Mean inhibition zone of positive control diameter (mm)
		AE	ME	AqE	AE	ME	AqE	Vancomycin
MT13W	Wall	12	11.5	-	15	16	-	16
PD23D	Doorknob	11	13	-	12	14	-	14
MD11B	Bedrail	13	14	-	16	18.5	-	23
PD19B	Bedrail	10.5	11	-	13	15	-	17
MT5W	Wall	13	14	-	14	21	-	14
AE48F	Floor	16	12.5	-	18	19	-	16
MD53F	Floor	14	16.5	-	21	23	-	14
SG17B	Bedrail	15	17	-	18	20.5	-	13
AE28B	Bedrail	10.5	11	-	11.5	13	-	15
SG3D	Doorknob	11	13	-	13.5	16	-	18
SG11B	Bedrail	0	11	-	10	12.5	-	20

Key: ME=Methanolic extract, **AE**= Acetone extract, **AqE**= Aqueous extract, - represents absence.

Table 4: Minimum inhibitory and minimum bactericidal concentration (MIC and MBC) of leaf extracts of *Carica papaya* on Methicillin resistant *Staphylococcus epidermidis*

Methicillin resistant *S. epidermidis* isolates	Wards Surfaces	MIC values (mg/mL)		MBC values (mg/mL)	
		Methanolic extract	Acetone extract	Methanolic extract	Acetone extract
MT13W	Wall	62.5	62.5	62.5	125
PD23B	Bedrail	31.3	62.5	62.5	62.5
MD11B	Bedrail	125	31.3	125	31.3
PD19B	Bedrail	62.5	31.3	62.5	31.3
MT5W	Wall	31.3	62.5	62.5	62.5
AE48F	Floor	125	125	125	125
MD53F	Floor	62.5	31.3	62.5	31.3
SG17B	Bedrail	125	62.5	250	125
AE28B	Bedrail	62.5	31.3	125	62.5
SG3D	Door	125	62.5	125	62.5
SG11B	Bedrail	31.3	62.5	62.5	125

Key: MIC= Minimum Inhibitory Concentration, **MBC**= Minimum Bactericidal Concentration.

Table 5: Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC and MBC) of seed extracts of *Carica papaya* on Methicillin resistant *Staphylococcus epidermidis*

Strains of *S. epidermidis*	Wards Surfaces	MIC values (mg/mL)		MBC values (mg/mL)	
		Methanolic extract	Acetone extract	Methanolic extract	Acetone extract
MT13W	Wall	62.5	125	125	125
PD23B	Bedrail	31.3	62.5	62.5	62.5
MD11B	Bedrail	31.3	62.5	62.5	125
PD19B	Bedrail	125	62.5	125	62.5
MT5W	Wall	31.3	62.5	31.3	62.5
AE48F	Floor	62.5	125	62.5	125
MD53F	Floor	31.3	62.5	62.5	125
SG17B	Bedrail	62.5	62.5	125	62.5
AE28B	Bedrail	62.5	31.3	125	62.5
SG3D	Doorknob	31.3	62.5	62.5	125
SG11B	Bedrail	62.5	125	62.5	125

Key: MIC= Minimum Inhibitory Concentration, **MBC**= Minimum Bactericidal Concentration.

Table 6: Statistical analysis between the activities of *Carica papaya* leaf and seed crude extracts

Turkey's multiple comparisons test	Leaf	Seed	P-values Acetone leaf and seed	P-values Methanol leaf and seed
Acetone vs. Methanol	0.6650	0.3753	0.0559[a]	0.0040[a]
Acetone vs. Water	< 0.0001	< 0.0001		
Methanol vs. Water	< 0.0001	< 0.0001		
Acetone vs. Positive control	< 0.0001	< 0.0001		
Methanol vs. Positive control	< 0.0001	0.0002		
Water vs. Positive control	< 0.0001	< 0.0001		

KEY: superscripts a = one sample t-test.

crude extract had the lowest MIC values (31.3 mg/mL) against PD23B, MD11B, MT5W, MD53F and SG3D and highest MIC values (125 mg/mL) against PD19B. Acetone seed crude extract gave highest MIC value (125 mg/mL) against MT13W, AE48F and SG11B and lowest MIC values (31.3 mg/mL) against AE28B.

The highest MBC values (125 mg/mL) of methanolic seed crude extract was observed against MT13W, PD19B, SG17B and AE28B while the lowest (31.3 mg/mL) against MT5W. Acetone seed crude extract gave highest MBC values (125 mg/mL) against MT13W, MD11B, AE48F, MD53F, SG3D and SG11B and the lowest values (62.5 mg/mL) against PD23B, PD19B, MT5W, SG17B and AE28B.

4. Discussion

The antibacterial activity of methanolic seed extracts of *C. papaya* against Methicillin resistant *S. epidermidis* is in support of the studies by Ayanfemi and Bukola (26) as well as Egbuonu (27) which showed seed extracts to be more effective than the leaf against all the organisms tested in their study. The variation of antibacterial activities of the different extracts depends on the polarity of the solvents used, concentrations of the compounds being extracted from each solvent and in addition to their extrinsic bioactivity and by their ability to dissolve or diffuse in the media used in the assay (28). Methanolic extracts were more effective in this study, this could be because it contained more phytochemicals (Flavonoids, Tannins, Terpenoids, Cardiac glycosides, Steroids, Alkaloids, Phenols and Triterpenes). However, water extracts exhibited activity against Methicillin resistant *S. epidermidis,* this could be attributed to the low quantity of phytochemicals (Phenols, Saponins, Tanins and Alkaloids) in the extracts. Plant extracts have the ability to either inhibit or completely kill the bacterial cell under study; this can be examined through the determination of minimum inhibitory and minimum bactericidal concentration of the active extracts.

The results of MIC and MBC from this study (250 to 31.3 mg/mL for leaf and 125 to 31.3 mg/mL for seed) were in line with the discoveries of Ayandele and Oluwaseun (29) which reported the MIC's and MBC's values of *C. papaya* leaf and seed extracts against many bacterial isolates including *Staphylococcus aureus* ranging between 200 to 150 mg/mL and 200 to 175 mg/mL respectively. However, the MIC's and MBC's values were higher compare to other studies reported by Mwesigwa (30) with MIC values ranging between 100 to 3.12 mg/mL against *E. coli* and Okunola (31) reported the MIC of *C. papaya* leaf extract against *E. coli, Salmonella, S. aureus* and *Streptococcus pyogens* ranged between 100 to 75 mg/mL. The high MIC's and MBC's values observed with extracts against test isolates might be an indication of low effectiveness or that the isolates have the potential for developing resistance to the bioactive compounds (32). Therefore, the high MIC's and MBC's values observed in this study with both the *C. papaya* leaf and seed extracts may be due to the fact that the organism used in this study were resistant isolates of *S. epidermidis*. The bioactivity of plant extracts is dependent upon its phytochemical constituents.

Conclusion

The research concluded that the leaf and seed methanolic and acetone crude extracts of *C. papaya* had an antibacterial activity against the Methicillin-resistant *S. epidermidis* isolated from KIU-TH wards surfaces. When compared, the methanolic crude extracts of both the leaf and seed had more activity than acetone crude extract. However, the aqueous crude extracts exhibited no activity against the resistant bacteria. This study recommended that *C. papaya* leaf and seed crude extract could be a potential source of novel antibiotic that can be utilized in the management of infections caused by *S. epidermidis*.

Conflict of interest

All authors have declared no conflict of interest

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