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EFFECT OF DIFFERENT EXTRACTION SOLVENTS ON THE ANTIMICROBIAL ACTIVITY OF PSIDIUM GUAJAVA (GUAVA) LEAVES AGAINST MULTI-DRUG RESISTANT BACTERIA IMPLICATED IN NOSOCOMIAL INFECTIONS

Fadahunsi Ilesanmi Festus[1+]
Oyeniran Opeyemi Goodness[2]
Ozabor Praise Temilade[3]

[1,2]*Food Microbiology and Biotechnology Unit, Department of Microbiology, University of Ibadan, Oyo State, Nigeria.*
[1]*Email: sanmifadahunsi@yahoo.com Tel: +2348062588059*
[2]*Email: oyenirangoodnessopeyemi@gmail.com Tel: +2347065271051*
[3]*Departmen of Microbiology, Osun State University, Osogbo, Osun State, Nigeria.*
[3]*Email: tn1praise@gmail.com Tel: +2347035235241*


(+Corresponding author)

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ABSTRACT

This present work was designed to investigate the effect of different extraction solvents on the antimicrobial activity of *Psidium guajava* leaves against some multidrug resistant bacteria in nosocomial infections. The MDR isolates such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Staphylococcus saprophyticus* and *Bacillus cereus* were obtained from Microbiology department culture collection of Department of Microbiology University of Ibadan Nigeria and their identities reconfirmed using biochemical methods. The antimicrobial activities of the different solvent extracts were tested using agar well diffusion method while the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts were monitored using the double fold dilution method. The results reconfirmed the identities of the bacteria as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Staphylococcus saprophyticus* and *Bacillus cereus*. Methanol extracts at a concentration of 160 mg/ml showed the highest inhibition zone of 10.0±0.1mm followed by ethyl acetate extract with 8.0±0.6mm inhibition zone against *E.coli* which were significantly different ($P<0.05$) while N-hexane, cold and hot water showed zero inhibition zones. Ofloxacin and DMSO. showed 20.0±0.and 0.00 mm inhibition zones respectively. This trend was observed at all the other concentrations. Methanol and ethyl acetate had MIC of 40.0 mg/ml for *E. coli*, *S. saprophyticus*, *P.aeruginosa* and *B. cereus* and they showed MBC of 80.0mg/ml for *E. coli*, *S. saprophyticus*, *P.aeruginosa* and *B. cereus* and MBC of 40.0 mg/ml for *S. Epidermidis*. Methanol and ethyl acetate extracts could be employed in the treatment of bacterial infections caused by MDR bacteria.

Contribution/Originality: The paper's primary contribution is finding that *Psidium guajava* (guava) leaf extracts can be used for the treatment of some multi-drug resistant bacteria implicated in nosocomial infections. Methanol and ethyl acetate extracts of the leaves possess the greatest antimicrobial activity due to the high sensitivity of the MDR organisms.

1. INTRODUCTION

The increasing problems of multi-drug resistant (MDR) bacteria is of great concern to both the clinicians and pharmaceutical industries and this has led to the utilisation of plants and herbs for their primary health care by approximately 80% of the world's population (Akinjogunla, Yah, Eghafona, & Ogbemudia, 2010; Pravin, 2006;

World Health Organization, 2008). In China, India, and Japan, had been reported to use natural plant extracts in enhancement of health since thousands of years ago. *Psidium guajava* belongs to the family Myrtaceae and originated from Central America and its fruits is widely consumed by millions of people in the tropical region of the world (El-Mahmood, 2009; Nair & Chanda, 2007). The genus consists of about 100 species of tropical shrubs that can be cultivated on different types of soil and is commonly known as guava in Nigeria, while the Hausa people also call it guava and the Yoruba and Igbo people identified it as gurfa and Gwaibwa respectively (El-Mahmood, 2009). The multi dimensional usages of the plants is highly documented, for instance its fruit is consumed fresh or processed into juice for human consumption because it contains tryptophan lysine, pectin, calcium, phosphorus, minerals and vitamin (Burkil, 1994) while its leaf, roots and bark are used in the therapy of wounds, boils, skin and soft tissue infectious site, gastro-enteritis, vomiting, toothaches, coughs, sore throat, oral ulcers, inflamed gums, leucorrhea, diabetes mellitus patient. In addition, it is reported that it could be used in the reduction of high level blood cholesterol, treatment of stomach ache, epilepsy, convulsions and upper respiratory tract infections (Bala, 2006; Lozoya et al., 2002). The anti malaria and antibacterial activities of guava leaf are dependent on its high total phenolic and essential oils contents (Morton, 2006). However Magno, Fontes, Gonçalves, and Gouveia (2015) reported that the leaf extract of guava demonstrated wide zone of inhibition against multidrug resistant *Proteus mirabilis*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Escherichia coli*,*Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Antibiotics resistance can be described as a phenomenon that occurs when an organism which was previously inhibited by an antibiotic agent has become insusceptible due to intrinsic features of their physiology or biochemistry (Levy & Marshall, 2004). According to Iwu, Duncan, and Okunji (1999) Incidents of epidemics resulting from drug resistant bacteria have constituted global threat to public health .It is also reported that the global emergence of multi-drug resistant bacteria is constituting a serious threat to the treatment of bacterial infections by limiting the effectiveness of antibiotic drugs (Hancock, 2005). Examples of multi-drug resistant bacteria include methicillin-resistant staphylococci,vancomycin-resistant enterococci and multi drug resistant gram-negative bacteria (Norrby, Nord, & Finch, 2005). Most of these multi drug resistant bacteria are highly pathogenic to humans and are capable of eliciting this phenomenon by forming biofilm and they include gram-negative and gram-positive bacteria. The formation of biofilm in a bacterium makes it 10–1000 times more resistant to the effects of antimicrobial agents. In addition other factors such as the presence of efflux pumps and multidrug resistance (MDR) proteins can contribute significantly to the intrinsic and acquired resistance in these bacteria (Oluwatuyi, Kaatz, & Gibbons, 2004). The most predominant live pathogens found in hospital wastes, (80-90%) is the genus *Bacillus* with *Staphylococci*, *Streptococci* whereas the most common pathogens is *Staphylococcus aureus Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* with varying numbers of other common nosocomial pathogens such as *Klebsiella Proteus and Enterobacter* species.

The increase in bacteria's resistance to conventional antibiotics has been reported in recent years thereby posing enormous public health concerns and making treatment very difficult and expensive. In view of this, plant extracts are being investigated as alternative remedies for the treatment of infectious diseases. and there is need to investigate the extraction solvent with the highest efficacy.

2. MATERIALS AND METHODS

2.1. Sample Collection

Fresh and healthy Guava leaves (*Psidium guajava*) were collected from the premises of Obafemi Awolowo hall, University of Ibadan and identified as *Psidium guajava* by the herbarium unit of the department Botany, University of Ibadan with the Voucher no. UIH 22824 The leaves were allowed to dry at room temperature for a month and pulverized into coarse powder using blending machine.

2.2. Source of Test Organisms

The multidrug resistant bacteria were collected from the culture collection of the Department of Microbiology, University of Ibadan and were maintained on nutrient agar slants at 4°C.

2.3. Purification of the Bacterial Isolates

The collected bacterial isolates were sub-cultured repeatedly to obtain pure cultures which were used for further study.

2.4. Antimicrobial Sensitivity Pattern

2.4.1. Preparation of Inoculums

The pure bacteria isolates were washed in phosphate buffer (0.05M pH7.0) and transferred into norrmal saline. Susceptibility was determined by agar disc diffusion method. and National Committee for Clinical and Laboratory Standard institute (C.L.S.I).One hundred µl of the suspension containing10^6 CFU/ml of bacteria was used to cover the surface of Oxoid- Mueller Hinton agar (Difco Laboratories, Detroit, Mich). plate using a sterile cotton swab and antibiotic disc such as Ceftazidime CAZ (30µg), Gentamicin GEN (10µg), Ciprofloxacin CPR (5µg), Ampicillin AMP (10µg), Chloramphenicol C (5µg) , Nalidixic acid NA (30µg), Trimethoprim W (5µg) , Tetracycline TE (30µg), Amoxicillin-clavulanic acid AUG (30µg), Nitrofurantoin NIT (30µg) (Oxoid ; Abtek Biologicals Ltd). were used to screen for the resistance of the bacterial isolates one after the other.

2.5. Confirmation of Identity

Biochemical characterizations such as Gram staining, Indole test: Catalase test: Citrate test: Oxidative test and Sugar Fermentation Test were used to re identify the multidrug resistant bacterial isolates.

2.6. Extraction Process

2.6.1. Preparation of Sample Extracts

The guava leaves were allowed to dry for a month at room temperature and grinded for different solvents extraction.

Aqueous extraction (hot): Seven hundred gm of the grounded dried sample was weighed, transferred into 4000ml (4L) of hot distilled water in 500 L Erlenmeyer flask The mixture was stirred at every two hours and allowed to stay for 24hours. The solvent (containing the extract) was collected using muslin bag and the filtrate was further filtered using NO1whatman filter paper and concentrated with the aid of rotary evaporator (Heidolph laborota 400 effiecient, Germany, model 517-01002-002) set at 50^0C, and further concentrated using a vacuum oven set at 50^0C with a pressure of 700mmHg.

Aqueous extraction (cold): Seven hundred gm of the grounded dried sample was weighed into 5,000ml (5L) of cold distilled water in a glass container and stirred at two hours interval with a glass rod and allowed to stay for 24h The solvent (now containing the extract) was filtered using No1 whatman filter paper and the filtrate concentrated with the aid of rotary evaporator (Heidolph laborota 400 effiecient, Germany, model 517-01002-002) set at 50^0C, and further concentrated using a vacuum oven set at 50^0C with a pressure of 700mmHg.

Methanol extraction: One hundred and five hundred (1500) g of the dried blended sample was transferred into 10L flask containing 5000ml (5L) pure methanol and stirred at two hrs interval and allowed to stay for 72hours. The solvent (now containing the extract) was collected using muslin bag and the filtrate was further filtered using No 1whatman filter paper . The filtrate obtained was concentrated using rotary evaporator (Heidolph laborota 400 efficient, Germany, model 517-01002-002) set at 40^{0}C, and further concentrate using a vacuum oven set at 40^{0}C with a pressure of 700mmHg.

2.7. Ethyl Acetate and N-Hexane Fraction

The solvent-solvent extraction method was used to determine the various fractions Fourty two gm(42g) of the methanol extract, was transferred into a 500ml flask containing 150mls of methanol and 100ml of distilled water was added and stirred. The mixture was transferred into a separating funnel and 200mls of pure hexane was added and carefully shaken. This solution obtained was allowed to stand for 10minutes in order to ensure proper partitioning of the two phases and the hexane layer (at the top) was collected after releasing the methanol/water layer. This process was repeated (about 8 times) until a clear layer was obtained for the hexane portion. The same procedure was repeated to obtain the ethyl acetate fraction. The various fractions obtained were then concentrated using the rotary evaporator and vacuum oven at 40^{0}C and the percentage yields for the various isolated fractions and the crude extracts obtained were calculated using the formula stated below (AOAC, 2000).

$$Percentage(\%)\ yield\ of\ the\ crude\ (w/w) = \frac{Weight\ of\ crude\ extract\ obtained}{\text{Weight of plant material used for the extraction}} X100$$

2.8. Qualitative Determination of Phytochemicals

The screening for the phytochemical constituents present in the guava leaf extracts was carried out according to the standard procedures described by Krishnaiah, Devi, Bono, and Sarbatly (2009) Phytochemicals such Alkaloids, Tannins, Saponins, Cardiac glycosides, Flavonoids,Terpenoids, Anthraquinones and Steroids. were determined qualitatively at the Department of Pharmaceutical Chemistry, University of Ibadan, Nigeria

2.9. Screening for Alkaloids

Two ml of 10% HCl was added to 1g of the extract in a test tube and the mixture was placed in a water bath for heating. The solution obtained was filtered and the pH was adjusted to 6. Half (0.5) ml of the filtrate was treated with a few drops of Mayer, Dragendroff and Wagner reagents separately and mixed. Observations of precipitate in the mixtures were indicative of positive tests for alkaloids. Creamy-white precipitate was positive for Mayer's reagent, orange-brown for Dragendroff' s reagent while reddish brown precipitate was positive for Wagner's reagent.

2.10. Screening for Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.11. Screening for Saponins

One gram of the extract was mixed with 5 ml distilled water in a test tube and vigorously shaken for 2 minutes. Frothing in the test extract shows the presence saponins.

2.12. Screening for Cardiac Glycosides (Keller-Killiani Test)

About 0.5 g was made up to 5ml with water, then 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added to it. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

2.13. Screening for Flavonoids

Five ml of 80% alcohol was added to 1g of the extract; the solution was boiled for 5minutes and then filtered while still hot. One ml of the filtrate was withdrawn from the mixture and a little amount (4-5 pieces) of magnesium turnings was added and steamed in a water bath. This was followed by treatment with a few drops of Conc. H_2SO_4. A red or intense red coloration depicts a positive result.

Screening for Screening for Terpenoids To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

2.14. Screening for Anthraquinones

About 0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

2.15. Screening for Steroids

To 0.5 g of extract dissolved in 2ml of chloroform 2ml of concentrated H_2SO_4 was added along the sides of test tube. The presence of steroids was indicated by colour change of the upper layer to red while the lower layer turned yellow.

2.16. Preparation of Inocula

One loopful of inoculum of each test organism from cryogenic vial was streaked on nutrient agar plate and incubated at 37°C for 24 hours.

2.17. Antimicrobial Assays

2.17.1. Preparation of 0.5 Mcfarland Standard

Eighty five(85ml) of 1% H_2SO_4 was transferred to 100ml volumetric flask containing 0.5ml of 1.75% $Bacl_2$ and was swirled. The volumetric flask was placed on magnetic stirring bar for 3-5 minutes for proper mixing and was examined visually for homogenisation and 7ml of the solution was dispensed into tightly capped transparent tube. The prepared Mcfarland standard was stored at room temperature (Olutiola, Famurewa, & Sonntag, 2000).

2.18. Extract Concentration for Antibacterial Testing

To obtain the required concentrations of the extract for antibacterial investigation a double fold dilution of the extract was carried out to obtain concentrations of 160 mg/ml, 80 mg/ml and 40 mg/ml in different test tubes.

2.19. Standardization of the Inoculum

The prepared Mcfarland was used for the standardization of the inoculum by comparing their turbidity with that of the Mcfarland standard. All the test organisms were subcultured on nutrient agar for 18-24 hours. Loopful of pure colonies on nutrient agar was transferred to test tubes containing 5ml of sterile 0.85% saline solution until turbidity matched that of 0.5 Mcfarland turbidity standard (1.5×10^8 CFU/mL).

2.20. Antibacterial Activity of the Extract against the MDR Bacteria

The agar well diffusion method and the National Committee for Clinical and Laboratory Standards institute were used to test for the antibacterial activity of the extracts against the MDR bacteria at different concentrations. Twenty millimetres of sterile Nutrient agar was dispensed aseptically into sterile Petri dishes and allowed to solidify for 15 minutes, The plates were labelled to represent the particular concentrations of the extracts

introduced into the wells. A sterile cotton swab was dipped into the standardised inoculums suspension and used to cover the surface of the sterile nutrient agar plate. Wells were bored using a sterile cork borer (7 mm diameter) and the extract (One hundred microlitres (100 µl) was dispensed into the wells. One hundred(100) µg/ml of Ciprofloxacin and Dimethyl suphuroxide were dispensed at the respective wells meant for positive control and negative controls respectively and incubated at 37°C for 24 hours. The antimicrobial activity was determined by taking measurements of diameter of the zones of growth inhibition and expressed in millimetres using a transparent ruler. The experiments were done using different extracting solvents of the Guava leaf mentioned earlier. against 6 multidrug resistant organisms. The experiment were carried out in duplicates.

2.21. Determination of MIC and MBC

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were estimated for each of the test bacteria in duplicates. Double fold dilution of the extract was carried out to obtain concentrations of 160 mg/ml, 80 mg/ml and 40 mg/ml in different test tubes and 1 ml of nutrient broth was added to each tube. One hundred µL of each of the test bacteria from a 24 hour-old culture (containing 10^8 Cfu/ml (0.5 McFarland's standard) was used to inoculate the tubes differently and incubated at 37°C for 24hours. A tube containing nutrient broth only was differently seeded with each of the test bacteria and incubated at the same temperature and time, served as control. The tubes were examined for bacterial growth based on turbidity. The minimum inhibitory concentration is the lowest concentration that completely inhibits the bacterial growth.

To determine the MBC, one ml of broth was collected from tubes, which did not show any growth and inoculated on sterile nutrient agar by streaking. The concentration at which there was no visible growth, was considered as the minimum bactericidal concentration (Doughari, 2006). MBC was defined as the lowest extract concentration at which 99.9% of the bacteria was killed. Each experiment was repeated twice.

2.22. Statistical Analysis

The obtained data were subjected to the one-way analysis of variance (ANOVA) and the results were expressed where appropriate as mean ± standard deviation. Differences between means of samples were compared using Duncan's multiple range tests at $P < 0.05$.

3. RESULTS

The source and the code of different organisms used is shown in Table 1. The bacterial isolates used in this study include *Staphylococcus epidermidis* (D2), *Escherichia coli* (D35) and *Staphylococcus saprophyticus* (D12) which were isolated from Hospital waste while *Bacillus cereus* (A3), *Pseudomonas aeruginosa* (5C) and *Proteus mirabilis* (5E) were isolated from hospital environmental.

Table-1. The source and the codes of different organisms used in this experiment.

Organisms	Code	Source
Staphylococcus epidermidis	D2	Hospital waste
Bacillus cereus	A3	Hospital Environment
Pseudomonas aeruginosa	5C	Hospital Environment
Proteus mirabilis	5E	Hospital Environment
Escherichia coli	D35	Hospital waste
Staphylococcus saprophyticus saprophyti	D12	Hospital waste

Source: All organisms were obtained from the culture collection center of the Department of Microbiology, University of Ibadan.

The yield of the different extraction solvents is presented in Table 2. The methanol , ethyl acetate and hexane fractions recorded yields of 20.53±0.2, 13.72±0.4, and7.12±0.3 percent respectively which were significantly

different from one another (P<0.05) while the Aqueous (hot and cold) extract showed yields of 6.40±0.1 and 5.70±0.5 respectively which were not significantly different. from each other.

Table-2. The yield of the different extraction solvents.

Extract	Yield (%)
Aqueous extract (hot)	6.40±0.1a
Aqueous extract (cold)	5.70±0.5a
Hexane fraction	7.12±0.3ab
Ethyl acetate fraction	13.72±0.4b
Methanol fraction	20.53±0.2c

Note: Means within a column followed by the same letter are not significant by Duncan's Multiple Range Test at 5% level of significance.

The result of screening for the presence of phytochemicals in the diiferent solvents extracts of guava leaves is presented in Table 3 .It was observed that terpenoids was present in all the extracts showing different quantities with the highest quantities seen in N- hexane and methanol extracts. High quantities of steroids were seen in N-hexane, methanol and ethyl acetate extracts but absent in the aqueous extracts (cold and hot water). High Saponins quantities were recorded in the ethyl acetate and methanol extracts but absent in cold and hot water extacts with lower quantity seen in N-hexane.The highest quantity of flavonoids was seen in methanol followed by ethyl acetate and and N-hexane but absent in hot and cold water extracts. However the highest quantities of tannins were recorded in ethyl acetate and methanol extracts followed by N-hexane and hot water extracts but absent in cold water extract. Cardiac glycosides was present in low quantities in all the extracts. Alkaloids were seen in low quantities in ethyl acetate, methanol, hot but absent in N-hexane and cold water extracts. The ethyl acetate, methanol extracts recorded the highest quantities of anthraquinones followed byN --hexane and hot water extracts but absent in cold water extract.

Table-3. Qualitative analysis of phytochemical constituents of *P. guajava* leaf using different solvent extracts.

Phytochemicals	N hexane	Ethyl acetate	Methanol	Hot water	Cold water
Terpenoids	+	++	++	+	+
Steroids	+	++	++	-	-
Saponins	+	++	++	-	-
Flavonoids	+	++	++	-	-
Tannins	+	++	++	+	-
Cardiac glycosides	+	+	++	+	+
Alkaloids	-	+	++	+	-
Anthraquinones	-	+	++	+	-

Key; ++ in Present high quantity - absent

The result of the gram reaction and the biochemical characteristics of the multi-drug resistant bacteria is presented in Table 4. The results showed that Isolate D35 was gram negative rod, utilized all the different sugars tested and showed negative reactions to coagulase, citrate and urease tests but showed positive reaction to methyl red, Voges proskeaur and indole tests. Isolate D35 is suspected to be *Escherichia coli*..Isolate D2 was gram positive cocci utilised all the different sugars tested, showed positive results to coagulase ,methyl red, Voges proskeaur, urease,citrate ,catalase and negative reactions to indole test, Isolate D2 is suspected to be *Staphylococcus epidermidis*.Isolate A3 was gram positive rod, showed positive reactions to all the different sugars tested and was negative to coagulase and urease tests but positive to methyl red ,Voges proskeaur, citrate and catalase and indole tests, Isolate A3 is suspected to be *Bacillus cereus*. Isolate 5E was a gram negative rod ,utilized glucose, fructose and xylose but did not utilize other sugars .It is negative to coagulase, indole tests and positive to methyl red ,Voges proskeaur, citrate, urease and catalase tests Isolate 5E is suspected to be *Proteus mirabilis* .Isolate 5C was a gram negative rod, utilised glucose, xylose and mannitol but did not utilize other sugars, it showed negative reactions to coagulase, urease, citrate, oxidase and catalase tests and positive reactions to methyl red, Voges proskeaur, indole

test. Isolate 5C is suspected to be *Pseudomonas aeruginosa*. Isolate D12 was a gram positive cocci, utilized all the different sugars tested and showed negative reactions to coagulase, urease, indole test tests and positive reactions to methyl red ,Voges proskeaur, citrate and catalase tests. Isolate D12 was suspected to be *Staphylococus saprophyticus*.

Table-4. The gram reaction and the biochemical characteristics of the multi-drug resistant bacteria.

	Gram THreacttion	Cat	Oxi	Ind	Cit	KOH	Suc	Mami	Glu	Fru	Malt	Lact	Sor	Xyl	Gal	Coag	Urease	MR	VP	MOT	Suspected organism
A3	+	+	Variable	-	+		+	+	+	+	+	+	+	+	+	-	-	+	+	+	*B. cereus*
D35	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	_	+	*E. coli*
5CE	-	+	+	-	+	+	-	+	+	-	-	-	-	+	-	-	-	-	-	+	*P. aeruginosa*
5EG	-	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	+	+	-	+	*P. mirabilis*
D2	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	*S. epidermidis*
D12	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	_	+	+	+	-	*S. saprophyticus*

Key: Ox= Oxidase, Ind= Indole, Cit= Citrate, Cat= Catalase, Gram= Gram stain reaction, KOH= Potassium hydroxide, Suc= Sucrose, Glu= Glucose, Gal= Galactose, Lact= Lactose, Malt= Maltose, Xyl= Xylose, Sor = Sorbitol, Mann= Mannitol, Coa= Coagulase, Fru= Fructose, VP= Voges Proskeaur, MR= Methyl red test, MOT= Motility, Ure= Urease

Table 5 shows the multidrug resistant pattern of the bacteria isolates It was observed that S. *epidermidis* (D2) was resistant to Ciprofloxacin, Oxacillin, Clindamycin and Sulphomethaxole (MAR index2/7) while *B.* cereus showed resistance to Ampicillin, Erythromycin and Gentamycin(MAR index3/14). However *S. saprophyticus*(D12) showed resistance to Ciprofloxacin, Oxacillin,Clindamycin and Sulphomethaxole and Tetracycline(MAR index2/7) and *E. Coli* (D35) showed resistance to Tetracycline, Chloramphenicol, Ampicillin, Ciprofloxacin, Nalidixic acid, Ertapenem, Cefotaxime and Sulphomethoxazole (MAR index 4/7)while *P. aeruginosa* (5C) demonstrated resistance to Ciprofloxacin, Gentamycin, Imipenem and Ceftazidime (MAR index 2/7) and *P. Mirabilis*(5E) resisted Ciprofloxacin, Gentamycin, Ampicillin, Chloramphenicol and Tetracycline. (MAR index 5/14)

Table-5. Multidrug resistant pattern of the bacteria.

Bacteria	Tet	C	Amp	Cip	Gen	Ipm	Caz	Na	Etp	Ctx	Sxt	Da	Oxa	Ery	MAR Index
S. epidermidis	S	S	S	R	S	S	S	S	S	S	R	R	R	S	2/7
B. cereus	S	S	R	S	R	S	S	S	S	S	S	S	S	R	3/14
S. saprophyticus	R	S	S	R	S	S	S	S	S	S	R	R	R	S	5/14
E. coli	R	R	R	R	S	S	S	R	R	R	R	S	S	S	4/7
P. aeruginosa	S	S	S	R	R	R	R	S	S	S	S	S	S	S	2/7
P. mirabilis	R	R	R	R	R	S	S	S	S	S	S	S	S	S	(5/14)

Note: R = resistance
S= sensitive
Cip: Ciprofloxacin, Oxa: Oxacillin, Da: Clindamycin, Sxt: Sulphomethoxazole, Tet: Tetracycline, Cpd: Cefpodoxime, Na: Nalidixic acid,Caz: Ceftazidime, Ctx: Cefotaxime
Amp: Ampicillin, Ery: Erythromycin, Gen: Gentamycin, C: Chloramphenicol, Etp: Ertapenem, Ipm: Imipenem, Amc: Amoxillin- clavilanote

Table 6 shows the antimicrobial activity of the different solvent extracts of *P.guajava* leaf against multidrug resistant bacteria at a concentration of 160 mg/ml. The results revealed that methanol extract showed the highest inhibition zone of 10.0±0.1mm followed by ethyl acetate extract with 8.0±0.6mm inhibition zone against *E.coli* which were significantly different (P<0.05) while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another.Ofloxacin and DMSO showed 20.0±0.and 0.00 mm inhibition zones respectively. It was also noted that methanol extract showed the highest inhibition zone 9.5±0.2 mm followed by ethyl acetate extracts with inhibition zone of8.0±0.6mm against *S.saprophyticus* which were significantly different (P<0.05) while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO showed 15.0±0.3 and 0.00mms inhibition zones respectively. However the ethyl acetate extracts showed the highest inhibition zones of 15.0± 0.5mm followed by an inhibition zone of 12.5±0.7mm showed by the methanol extract against *P.aeruginosa*, which were significantly different (P<0.05) while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO. showed20.0±0.and 0.00 mm inhibition zones respectively. In addition B.cereus was susceptible to ethyl acetate extract showing inhibition zone of 13.5±0.8mm followed by methanol extract with an inhibition zones of 13.0 ±0.1 which were not significantly while the while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO showed 18.0±01. and 0.00mm inhibition zones respectively. The methanol extract had the highest inhibition zone of 18.0±0.3mm followed by ethyl acetate extract with zones of inhibition of 13.0±00mm against *S.epidermidis* while the N-hexane ,cold and hot water extracts showed zero inhibition. Ofloxacin and DMSO showed 20.0±0.1and 0.00mm inhibition zones respectively. P.mirabilis was inhibited only by methanol extract with an inhibition zone of 11.0±0.4mm while the other extracts showed zero inhibition zone while ofloxacin and DMSO showed 25.0±01. and 0.00mm inhibition zones respectively.

Table-6. Antimicrobial activity of the different solvent extracts of *P.guajava* leaf against multidrug resistant bacteria at a concentration of 160 mg/ml.

Bacteria	ME	EAE	NHE	HWE	CWE	OF	*DMSO*
E. coli	10.0±0.1^{c}	8.0±0.6^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	20.0±0.2^{d}	0.0±0.0^{a}
S.saprophyticus	9.5±0.2^{c}	8.0±0.2^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	15±0.3^{d}	0.0±0.0^{a}
P. aeruginosa	12.5±0.7^{b}	15.0± 0.5^{c}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	18.0±0.1^{d}	0.0±0.0^{a}
B. cereus	13.5±0.8^{b}	13.0±0.1^{b}	0.0±0.0^{a}	0.0±.0.0^{a}	0.0±0.0^{a}	15.0±0.3^{c}	0.0±0.0^{a}
S.epidermidis	18.0±0.3^{c}	13.0±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	20.0±0.1^{d}	0.0±0.0^{a}
P. mirabilis	11.0±0.4^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±.0.0^{a}	25.0±0.1^{c}	0.0±0.0^{a}

Key: ME: Methanol extract; EAE: Ethyl acetate extract; NHE: N- hexane extract; HWE: Hot water extract;CW: Cold water extract; OF: Ofloxacin

Means within a column followed by the same letter are not significant by Duncan·s Multiple Range Test at 5% level of significance. $p \leq 0.05$

The result of the antimicrobial activity of different solvent extracts of *P.guajava* leaf against multidrug resistant bacteria at a concentration of 80mg/ml is shown in Table 7 The results revealed that methanol extracts showed the highest inhibition zone of 8.0±0.1mm followed by ethyl acetate extracts with 7.0±0.6mm inhibition zone against *E.coli* which were significantly different (P<0.05) while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO. showed20.0±0.and 0.00 mm inhibition zones respectively.It was also noted that methanol extract showed the highest inhibition zone 6.4±0.3 mm followed by ethyl acetate extracts with inhibition zone of 6.0±0.1mm against *S.saprophyticus* which were not significantly different (P<0.05) while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO showed 15.0±0.3 and 0.00mm zones inhibition zones respectively. However the ethyl acetate extracts showed the highest inhibition zones of 12.0± 0.7mm followed by an inhibition zone of 11.0±0.3mm showed by the methanol extract against *P.aeruginosa*, which were significantly different (P<0.05) while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO.

Showed18.0±0.2.and 0.00 mm inhibition zones respectively. In addition B.cereus was susceptible to ethyl acetate extract showing inhibition zone of 11.0±0.3mm followed by methanol extract with an inhibition zones of 10.8 ±0.1 which were not significantly different while the while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO showed 15.0±04 and 0.00mm inhibition zones respectively..The highest inhibition zone of 16.5±0.3mm was shown by methanol extraxt followed by ethyl acetate extract with zones of inhibition of 14.8±0.4mm against *S.epidermidis* while the N-hexane ,cold and hot water extracts showed zero inhibition. Ofloxacin and DMSO showed 20.0±0.3and 0.00mm inhibition zones respectively. P.mirabilis was inhibited only by methanol extract with an inhibition zone of 6.8.0±0.3mm while the other extracts showed zero inhibition zone while ofloxacin and DMSO showed 25.0±01. and 0.00mm inhibition zones respectively.

Table-7. The antimicrobial activity of different solvent extracts of *P. guajava leaf* against multidrug resistant bacteria at a concentration of 80mg/ml.

Bacteria	ME 80mg/m	EAE 80mg/ml	NHE 80mg/ml	HWA 80mg/ml	CWA 80mg/ml	OF	DMSO
E. coli	8.0±0.1^{c}	7.0±0.1^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	20.0±0.6^{d}	0.0±0.0^{a}
S.saprophyticus	6.4±0.3^{b}	6.0±0.1^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	15.0±0.8c	0.0±0.0^{a}
P. aeruginosa	12.0±0.7^{c}	11.0±0.3^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	18,0±0.2^{d}	0.0±0.0^{a}
B. cereus	11.5±0.1^{b}	10.2±0.3^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	15.0±0.4^{c}	0.0±0.0^{a}
S. epidermidis	16.5±0.2^{c}	14.8±0.4^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	20.0±0.3^{d}	0.0±0.0^{a}
P mirabilis	6.8±0.3^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	25.0±0.1^{c}	0.0±0.0^{a}

Key: ME: Methanol extract; EAE: Ethyl acetate extract; NHE: N- hexane extract; HWE: Hot water extract; CW: Cold water extract; OF: Ofloxacin
Means within a column followed by the same letter are not significant by Duncan·s Multiple Range Test at 5% level of significance.

The result of the antimicrobial activity of different solvent extracts of *P.guajava* leaf against multidrug resistant bacteria at a concentration of 40mg/ml is shown in Table 8 The results revealed that methanol extracts showed the highest inhibition zone of 6.0±0.2mm followed by ethyl acetate extracts with 4.5±0.2mm inhibition zone against *E.coli* which were significantly different (P<0.05) while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO showed 20.0±0.6.and 0.00 mm inhibition zones respectively.It was also noted that methanol extract showed the highest inhibition zone 6.2±0.0 mm followed by ethyl acetate extract with inhibition zone of 5.8±0.0mm against *S.saprophyticus* which were not significantly different (P<0.05) while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO showed 15.0±0.0 and 0.00mm zones inhibition zones respectively. However the ethyl acetate extracts showed the highest inhibition zones of 8.0± 0.40mm followed by an inhibition zone of 6.5.0±0.0mm showed by the methanol extract against *P.aeruginosa*, which were significantly different (P<0.05) while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO showed18.0±0.0.and 0.00 mm inhibition zones respectively. In addition B.cereus was susceptible to methanol extract showing inhibition zone of 10.0±0.2mm followed by methanol extract with an inhibition zones of 8.5±0.1 which were significantly different while the while N-hexane, cold and hot water extracts showed zero inhibition zones which were not significantly different from one another. Ofloxacin and DMSO showed 15.0±02 and 0.00mm inhibition zones respectively.The highest inhibition zone of 9.5.0±0.3mm was demonstrared by the methanol extract followed by ethyl acetate extract with zones of inhibition of 8.5.0±01mm against *S.epidermidis* while the N-hexane ,cold and hot water extracts showed zero inhibition. Ofloxacin and DMSO showed 20.0±0.3and 0.00mm inhibition zones respectively. P.mirabilis was inhibited only by methanol extract with an inhibition zone of 5.0±0.3mm while the other extracts showed zero inhibition zone while ofloxacin and DMSO showed 25.0±04. and 0.00mm inhibition zones respectively.

Table-8. The antimicrobial activity of different solvent extracts of *P. guajava leaf* against multidrug resistant bacteria at a concentration of 40mg/ml.

Organism	ME (40mg/ml)	EAE (40mg/ml)	NHE (40mg/ml)	HWA (40mg/ml)	CWA (40mg/ml)	OF	DMSO
E. coli	6.0±0.2 ^{c}	4.50.2±^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	20.0±0.6^{d}	0.00.0±^{a}
S.saprophyticus	6.2±0.0^{b}	5.8±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	15.0±0.0^{c}	0.0±0.0$_{a}$
P. aeruginosa	8.0±0.4^{c}	6.5±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0a	18.0±0.2^{d}	0.0±0.0^{a}
B. cereus	10.0±0,2^{c}	8.5±0.1^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	15.0±0.0^{d}	0.0±0.0^{a}
S. epidermidis	9.5±0.1^{c}	8.5±0.9^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	20.0±0.0^{d}	0.0±0.0^{a}
P mirabilis	5.0±0.2^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	25.0±0.4^{c}	0.0±0.0^{a}

Key: ME: Methanol extract; EAE: Ethyl acetate extract; NHE: N- hexane extract; HWE: Hot water extract; CW: Cold water extract; OF: Ofloxacin
Means within a row followed by the same letter are not significant by Duncan's Multiple Range Test at 5% level of significance.

The results of MIC and MBC values are shown in Table 9. The result revealed that methanol and ethyl acetate extracts had the same MIC value of 40.0 mg/ml for *E. Coli*, *S. Saprophyticus*, *P.aeruginosa* and *B. cereus* and the same MIC value of 20.0 mg/ml for *S. epidermidis* while methanol extract had MIC value of 40.0 mg/ml for *P.mirabilis* and ethyl acetate extract had 00.0 mg/ml *P.mirabilis* which were significantly different However methanol and ethyl acetate extracts had the same MBC values of 80.0mg/ml for *E. Coli*, *S. Saprophyticus*, *P.aeruginosa* and *B. cereus* and the same MBC values of 40.0 mg/ml for *S. epidermidis* while methanol extract had MBC value of 80.0 mg/ml for *P.mirabilis* and ethyl acetate extract had 00.0 mg/ml *P.mirabilis* which were significantly different.

Table-9. MIC and MBC values for methanol and ethyl acetate extracts.

Name of organisms	MIC (Methanol) mg/ml	MIC (Ethyl acetate) mg/ml	MBC (Methanol) mg/ml	MBC (Ethyl acetate) mg/ml
E .coli	40.0±0.5^{a}	40.0±0.0^{a}	80.0±0.1^{a}	80.0± 0.1^{a}
S. saprophyticus	40.0 ± 0.2^{a}	40.0±0.6^{a}	80.0±0.3^{a}	80.0±0.5^{a}
P.aeruginosa	40.0 ±.0.7^{a}	40.0±0.1^{a}	80.0±0.0^{a}	80.0±0.1^{a}
B. cereus	40.0±.00.1^{a}	40.0±0.2^{a}	40.0±0.4^{a}	80.0±0.3^{a}
S. epidermidis	20.0± 0.3^{a}	20.0±0.0^{a}	80.0±0.2^{b}	40.0±0.3^{a}
P.mirabilis	40.0.0±0.3^{b}	0,0±0.0^{a}	80.0±0.1^{a}	0.0±0.0^{a}

Note: Means within a column followed by the same letter are not significant by Duncan's Multiple Range Test at 5% level of significance.

4. DISCUSSIONS

This present work provides information on the effect of extraction solvents on the antimicrobial activity of guava leaf extracts against multi- drug resistant microorganisms implicated in nosocomial infections It was revealed that different phytochemicals are present in the different extracts of *Psidium guajava*. The variation in the quantity of phytochemicals present in the different solvent extracts is dependent on the solvent used in the extraction process, this observation is similar to the findings of Bishnu, Sunil, and Anuja (2001). There are documented information on the production of phytochemicals by the plants (Fawole et al., 2010; Yoshida et al., 2008; Zhang & Lin, 2008). The plants produce these chemicals to protect themselves against hazards but recent research discovered that they can also used in the treatment of chronic diseases such as heart disease, cancer, hypertension, diabetes and other medical conditions Thus medicinal plants are becoming relevant in pharmaceuticals and neutraceuticals (Sen & Batra, 2012).

The isolation of multi- drug resistant microrganisms from environmental and hospital wastes had earlier been reported by Zhang. et al. (2009). The ability of these organisms to survive in these environments is due to their ubiquity which is derived from their adaptability capability. In addition they were able to exhibit multidrug resistance characteristics by forming biofilm and by the possession of efflux pumps and multidrug resistance (MDR) proteins which contribute significantly to the intrinsic and acquired resistance in these bacteria (Oluwatuyi et al., 2004).

The variation in the degree of inhibitions of the multidrug resistant bacteria by the different solvent extracts could be ascribed to difference in their genetic constitution and quantity of phytochemicals present in them

(Aboaba, Ezeh, & Anabuike, 2011). These phytochemicals in plant extracts enhance their antimicrobial activity (Zhang & Lin, 2008) by damaging the DNA or inhibit the synthesis of proteins in these pathogens (Fatope, 1995). Therefore the presence of these compounds in higher amounts in the *P. guajava* extract may explain its suitability in the development of phyto-medicines or as a source of vital compounds for drug development.. The relatively higher inhibitory patterns demonstrated by methanol and ethyl acetate extracts even at lower concentrations may be due to the presence of higher quantity of phytochemicals which act by inhibition of electron transport, protein translocation, phosphorylation steps, and other enzyme-dependent reactions, followed by an increase in plasma membrane permeability and finally ion leakage from the bacterial cells (Walsh et al., 2003) and it can be suggested that the methanol and ethyl acetate extracts possess outstanding solubility capacities for different phytochemicals (Walsh et al., 2003). The lower inhibitory zones demonstrated by the other solvent extracts may have emanated from the possession of little quantity of phytochemicals. The inhibition zones shown by the different extracts in this study which were greater than 10mm could be considered effective against the test organisms as stated by Nand, Drabu, and Gupta (2012). There are several reports that showed that the activities of extracts were largely dependent on the types of extraction solvent used as observed in this work and is in conformity with the reports of (Usman, Usman, & Mainasara, 2015). From the susceptibility pattern of the tested bacteria to different solvents extracts it could be inferred that the extracts have broad spectrum activity against both Gram positive and gram-negative bacteria (Cowan, 1999). A few studies by Gibbons, Oluwatuyi, Veitch, and Gray (2003); Braga et al. (2005); Dickson, Houghton, Hylands, and Gibbons (2006) and have reported that plant extracts can enhance the *in vitro* activity of certain antibiotics against strains of MDR *Staphylococcus aureus* and other pathogens.

MIC and MBC values obtained in this study showed that the MIC values are lower than the MBC values and this is a revelation that the extracts are inhibitory at lower concentration but bactericidal at higher concentration (Rahman, Salehin, Jamal, Pravin, & Alam, 2011). In addition, the ratios of MBC/MIC were less or equal to 4 which indicates that the solvent extracts are bactericidal (Tepe, Daferera, Sökmen, Polissiou, & Sökmen, 2004) MIC data are very important because they can be used as reference point in the treatment of bacterial infections (Aboaba et al., 2011) and assessment of the inhibitory potential of the plant extracts .The therapeutic use of plants especially as antimicrobials has been reported by many scientists (Cowan, 1999; Nostro, Germarno, D'Angelo, Marino, & Canatelli, 2000). Antimicrobial substances of plant origin demonstrate huge therapeutic potential because incidents of bacterial resistance to them is lesser reported as compared to synthetic antimicrobials (Cowan, 1999; Nostro et al., 2000; Raskin et al., 2002).

The results indicates that Methanol extracts are more effective than N-hexane and Aqueous extracts of *P. guajava leaf*, it possesses the greatest antimicrobial ability which can be used against multidrug resistant organisms. From the results obtained in this study it could be concluded that the multi-drug resistant bacteria were significantly sensitive to the Methanol and Ethyl acetate extracts of guava leaves hence could be employed for the treatment nosocomial infections caused by MDR bacteria.

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